

ORGANIZATION, REPRODUCTION AND INHERITANCE IN PEDIASTRUM.

(PLATES V. AND VI.)

By R. A. HARPER.

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I have discussed in previous papers ('08, '12) the problems of organization as seen in strictly cœnobic plants in which the colony shows little or practically no differentiation in either the structure or the functions of its cells. In *Pediastrum* we have a type in which at least the incipient steps in differentiation can be recognized.

The margins of the flat plate-shaped cell colonies are in some species quite entire, in others more or less lobed or toothed, and present the problems of the development and inheritance of specific and differentiated form in plants at a relatively critical stage. We have here the first beginnings of cell, and, we may say, tissue differentiation. In such cœnobes as most species of *Spirogyra*, *Hydrodictyon*, *Gonium* and others, though the colonies have definite and probably adaptive structure, the cells are all alike in form and function, but in certain species of *Pediastrum* the lobed peripheral cells differ markedly from the interior cells of the colony. In other species the lobing is almost equally developed in all the cells. The genus thus presents us with the processes of differentiation in varying degrees of expression in what are plainly rather closely related species.

I have also discussed elsewhere ('16) the interrelations of the cells in the eight- and sixteen-celled colonies of *Pediastrum Boryanum* as giving the basis for a definite conception of plant types and the comparison of this species with the other members of the group brings out still more clearly the idea of biological form types as I have discussed it there. The group is also well adapted to illustrate the relations of heredity and environment in morphogenetic

processes and to give evidence as to the possible mode of origin and interrelations of different types.

The shape of the cell is the main basis of group distinctions in the genus. As Braun ('55) first pointed out, the number of cells in the colony has been shown not to be diagnostic of species or larger aggregates. Fig. 21 shows two daughter colonies, one with sixteen and one with thirty-two cells, both of which came from the same thirty-two-celled mother colony. The shape of the cells of a given species varies in minor details of proportion about a norm for the species. The form of the cells of different species are of more fundamentally diverging types.

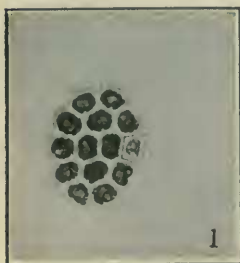


FIG. 1.



FIG. 2.

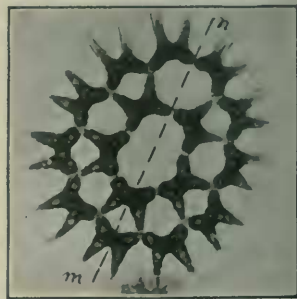


FIG. 3.

FIG. 1. *Pediatrum integrum*. Cells almost without spines or lobes. The colony shows only fourteen cells but it is possible, as is common for this species, that it is partially two layered. $\times$ about 225.

FIG. 2. *P. simplex*. Cells with one spine. Configuration 1 + 7. $\times$ about 150.

FIG. 3. *P. clathratum*. Cells with two very long and slender peripheral and two shorter basal lobes, large intercellular spaces. Configuration 5 + 11. Colonies bilaterally symmetrical about the axis *m*, *n*. $\times$ about 350.

It is a question of first importance morphogenetically whether these cell forms are hereditary or whether they are newly achieved under the stimulus of the intercellular environment in each generation. It is plain that the form is not inherited directly as such since the two-spined cells do not divide in such a way as to produce at once two equivalent two-spined daughter cells.

The main distinctions in the group are between forms whose cells have no spinous processes or very rudimentary ones, and those whose cells have more or less developed spines. These distinctions

have been regarded as of subgeneric rank and are the basis of the *integrum* (Fig. 1), Monactinia or *simplex* (Fig. 2), the Diactinia or two-spined (Fig. 3), the Triactinia or three-spined and the Tetractinia or four-spined series. A tendency to the formation of a four-spined type of cell is shown in the species *P. biradiatum* Meyen, and the peripheral cells of *P. tricornutum* Borge are described as having three spines. Braun ('55) recognized the diagnostic value of cell form in making four sections of the genus, the Monactinia, *P. Simplex*, Anomopodium, *P. integrum* (Näg.), the Diactinia, *P. Boryanum*, etc., and the Tetractinia, *P. Ehrenbergii*.

A further class of differences which has been given specific rank is found in the degree of similarity in form between all the cells of the colony. In *P. Boryanum* (Fig. 4) the interior cells are quite different in form from the peripheral series, while in *P. integrum* (Fig. 1), on the one hand, and *P. clathratum* (Fig. 3) on the other, the cells are very much alike throughout the colony.

It seems natural to assume that such forms as *P. integrum* (Fig. 1) with its oval cells, sometimes with two papillæ, but with no striking form differentiations, represent the more primitive species, though perhaps *P. integrum* itself is only an environmental form as I am suggesting in another paper, and that evolution has progressed toward the *simplex* type with one spine on the one hand, and the two-spined type on the other. The tendency to the development of spines more strongly on the peripheral cells probably came first (*P. Boryanum*, Fig. 4) and later the development of the strongly four-lobed form in all the cells of the colony (*P. clathratum*, Fig. 3). Positive evidence that the evolution of the group has followed this course is, however, lacking. The origin of the tendency to produce spines is also not obvious. We shall find a direct relation between the cell form and the intercellular relations in the colony in comparing the colonies of *P. Boryanum* (Fig. 4) and *P. asperum* (Fig. 5) and we have evidence for the assumption that the result of a direct environmental influence has been transformed into an inherited cell character in such cases but no support for such an hypothesis is found in the three-spined cells of *P. tricornutum* Borge or the four-spined cells of *P. biradiatum* Meyen.

As noted, the number of cells in a colony has been shown to

have relatively slight diagnostic value for the species. *P. asperum* like *P. Boryanum* may have eight, sixteen, thirty-two or sixty-four cells. Still certain species tend toward higher and others toward lower cell numbers. *P. Ehrenbergii* commonly occurs with four, eight, or sixteen cells. The rule that the number of cells in any species is a multiple of two, based as it is on the law of cellular bipartition, is very universally maintained and has long been recognized. Individuals with fifteen cells instead of sixteen cells or

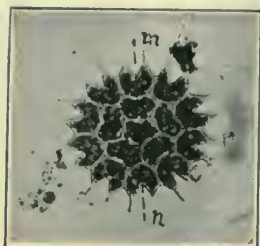


FIG. 4.

FIG. 4. *Pediastrum Boryanum*. Peripheral cells with two spines very slender for the species. Interior cells with merely a reëntering angle to indicate the spines. No intercellular spaces. Configuration $1+5+10$. Colony bilaterally symmetrical about the axis *m*, *n*. $\times$ about 300.

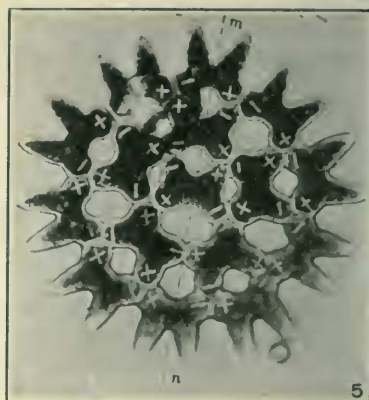


FIG. 5.

FIG. 5. *P. asperum*. Cells with two fairly long peripheral spines and two short, blunt basal lobes. Intercellular spaces well developed, the curves which bound them suggesting the origin of the cell lobes by catenoidal deformation. Configuration $1+5+10$. Colony bilaterally symmetrical about the axis *m*, *n*. Possible polarities of the cells suggested by the plus and minus signs. $\times$ about 600.

thirty-one instead of thirty-two cells occur, but as Nitardy ('14) has emphasized, they are great rarities and may be properly regarded as abnormal. The figure of *P. integrum* given shows only fourteen cells (Fig. 1).

The relative development of the spine is directly correlated with that of the intercellular spaces and we may consider first the organization of a form with well-developed intercellular spaces. Perhaps

the most abundant species as I have found them, next to *P. Boryanum* Kg., is *P. asperum* (Figs. 5 and 6). *P. asperum* has been regarded by many as a form of *P. Boryanum*. Whether or not it is to be considered a good species, the forms as I have found them reproduce the type so far as cell form, involving as it does the characteristic intercellular spaces, is concerned with a high degree of constancy. I have never seen a colony with the *asperum* cell form produced from a *Boryanum* parent nor a colony without intercellular spaces from an *asperum* parent.

I have been able to study and photograph *P. asperum* in all stages of the vegetative growth and reproduction of the colonies and it may well serve to illustrate the two-spined forms with well-developed intercellular spaces and cells similar throughout the colony in contrast with *P. Boryanum* with intercellular spaces small or wanting and interior cells only slightly or even not at all two-spined, as I have already described it ('16).

ARRANGEMENT OF THE CELLS AND INTERCELLULAR SPACES IN THE SIXTEEN-CELLED COLONIES OF *P. ASPERUM*.

The spatial interrelations of the cells are essentially the same in the typical sixteen-celled colony of *P. asperum* as in the typical sixteen-celled colony of *P. Boryanum*, as I have described it elsewhere ('16), and as it has long been known in the literature. But in *P. asperum* the cells are all very much alike in form, the interior cells having only slightly shorter lobes than those on the periphery of the colony. We may consider first the sixteen-celled colony, which is perhaps the most common, though 8-, 32-, 64- and 128-celled colonies are found. I shall leave the colonies with 32, 64, 128, etc., cells without discussion at this time, since certain further elements in morphogenesis implied in the larger number of units are involved which I shall take up in a later paper. A common arrangement in the thirty-two-celled colony is $1 + 6 + 10 + 15$, as noted by Braun ('55). The presence of six cells in series II. of the thirty-two-celled colony as compared with the five in series II. of the sixteen-celled colony introduces quite fundamentally different relations between the oblong four-lobed form of the cells and the natural surface tension factors in such groups.

As in *P. Boryanum*, the general arrangement of the cells in the most common form of sixteen-celled colony of *P. asperum* is one in the center with five around this and two cells in the third or outer series, the $1 + 5 + 10$ group, as Nägeli and Braun called it. Nägeli ('49, p. 93) quite fully described these conditions for the group and by comparing the areas of the concentric circles, assuming the radial diameters of the cells are the same, showed geometrically that these cell numbers are what he calls the "most natural," that is, the cells so arranged come the nearest to filling the spaces in the concentric circles as well as being the ones most commonly found. I have described this arrangement ('16) as coming the nearest to that of a least-surface configuration for such a group of rounded cells lying in one plane. As I shall further discuss in another connection, Nägeli is, however, probably mistaken, at least for *P. Boryanum*, as Braun's figures ('55) show, in saying that for eight cells the arrangement $2 + 6$ is much less common than the "more natural" arrangement $1 + 7$.

I shall follow the same method of numbering the cells here as in the case of *P. Boryanum* (Fig. 25, and '16, Fig. 1b), making the central cell No. 1 and proceeding outward as shown in the diagram (Fig. 7). The colony is bilaterally symmetrical as in *P. Boryanum*, the axis bisecting cells 1, 4, 7 and 12 and passing through the surface of contact of cells 2 and 6. For describing the structure of the colony more conveniently we may here, as in the case of *P. Boryanum*, call the ends of this axis of the colony its *m* and *n* poles respectively.

The central cell is in contact with five cells. The cells of the second series are each in contact with six cells and the cells of the outer series are alternately in contact with three and four cells. The cell walls meet in threes on the principle of least surfaces, except where cells 2 and 6 are in contact as a pair right and left of the axis of the colony. In *P. Boryanum* this grouping of intersections is universal in the more regular sixteen-celled colonies and the paired contact of cells 2 and 6 is one of the most obvious differences between the two species in their intercellular relations.

If the tips of the lobes of a cell of series II. be connected in serial order, g^1, g^2, d^3, d^2 , by straight lines, we have a trapezoid with

its longest side away from the center of the colony. In the case of the central cell we have a trapezoid with its longest side connecting the apices of the basal lobes. As in *P. Boryanum*, five of the outer cells stand radially outward from the five cells of series II. and five stand radially opposite the surfaces of contact of the cells of series II.

Cells eight and sixteen, nine and fifteen, ten and fourteen, eleven and thirteen are paired to the right and left of the axis of the col-

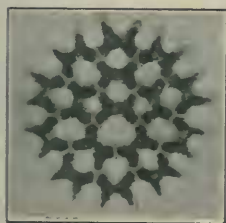


FIG. 6.

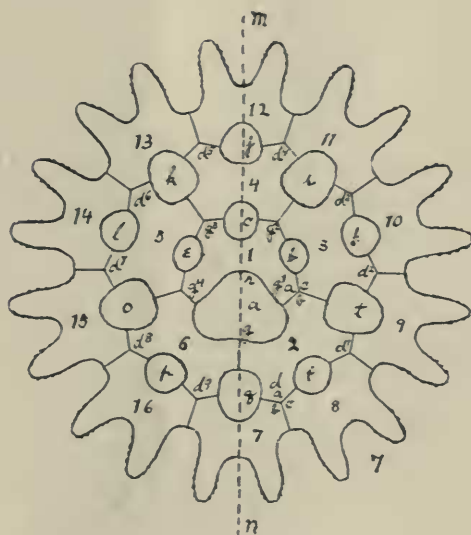


FIG. 7.

FIG. 6. *Pediatrum asperum*. Very typical colony organization as described for Fig. 5. This colony served as the model for the type diagram for the species given in Fig. 7. $\times$ about 325.

FIG. 7. Type diagram for *P. asperum*. Angles of intersection of all the walls made 120° . Inter-cellular spaces and peripheral spines sketched free-hand from the colony shown in Fig. 6. Numbering of cells and lettering of angles as in diagram of *P. Boryanum*, fig. 35.

ony. The adaptive and symmetrical adjustment by which, with the products of cellular bipartition to be grouped, we get five instead of six cells in series II., thus leaving ten for series III., which can be so spaced as to place one cell opposite each cell of series II. and one cell opposite the surface of contact of each pair of cells in series

II., is just as conspicuous here as in the case of *P. Boryanum*. The delicacy of the pressure and contact responses which prevents the placing of six, the normal least-surface number, around the central cell, thus leaving only nine for the peripheral series, and resulting in quite asymmetric contact relations between series II. and III., affords good evidence of the efficiency of cellular interactions in the production of morphogenetic adjustments.

The four-lobed form of the cells and the spacing of the five cells of series II. about the central cell and the ten cells of series III. about the five cells of series II., leave free intercellular areas and the symmetrical distribution of these areas in such fashion as to maintain most perfectly the rigidity of the colony and the equality of the cells has led to the development of one of the very obvious differences between *P. asperum* and *P. Boryanum*. This is perhaps the most conspicuous difference between the two species. The cells instead of being in contact on the entire extent of their adjacent surfaces show a series of intercellular openings which perforate the plate-shaped colony like the holes in a sieve. An intercellular space is formed between each pair of contact walls of the sixteen cells. They are of such form and are so placed that the interior cells of the colony have the four-lobed form of the peripheral cells. The cells of the whole colony are thus, as noted above, much less differentiated in form than those of the colony of *P. Boryanum*. The inner cells differ from the outer cells, broadly speaking, only in that their peripheral lobes are blunted and shortened where they meet the inner lobes of the outer series. *P. asperum* conforms much more nearly to the definition of a cœnobe as a colony of cells quite similar in form and function.

One might conclude that we have here a simpler type in which cell differentiation has not yet gone so far as in *P. Boryanum*. I am inclined to the view, however, that in reality *P. asperum* is the more specialized type and expresses more fully the form-determining tendencies which have led to the development of the spines or lobes on the peripheral cells of the colony of *P. Boryanum*.

As we shall find from a study of the method of reproduction in the colony, the cells all inherit alike the tendency to the four-lobed form. This is obvious even in the interior cells of the colony

of *P. Boryanum*. They all show a reëntering angle on their peripheral sides. In *P. asperum* this tendency to the four-lobed form has come more fully to expression, overcoming to quite a degree the adhesion of the cell surfaces so that each cell may assume the form to which its inherited tendencies predispose it. The possible origin of this tendency in the intercellular relations of such groups of cells, I shall discuss later.

In form these intercellular spaces vary from youth to the adult reproductive condition, as will be noted later in discussing reproduction. They also show some degree of variation in different individuals of apparently the same stage of development. In their curved outlines they express very fully the tensions existing in the protoplasm of the cells and the contact relations of the cells in the colony. In colonies with atypical cell arrangement, they may become highly varied in different regions, expressing fully the different and irregular tensions set up in such abnormal or subnormal groupings (Figs. 26-28).

In number, form and position these intercellular spaces form two series, four between the central cell and series II. and ten between series II. and III., the outer series. Of the four intercellular spaces of the inner series two, *a* and *c* (text-fig. 7) lie on the axis of the colony, and the other two, *b* and *e*, are symmetrically placed to the right and left. These four spaces are of three forms and sizes, *a*, the largest, is broadly triangular to shield-shaped (Figs. 5, 6); *c*, the second in size, is roughly an asymmetrical ellipsoid with its greater convexity toward the central cell, and *b* and *e* are still smaller and more or less symmetrical ellipsoids with pointed poles, somewhat lemon-shaped.

The outer series consists of five larger, ovoid to shield-shaped spaces, *i*, *k*, *o*, *t*, and *q*, and alternating with them five smaller spaces, *f*, *h*, *j*, *l* and *p*. One of the larger spaces, *q*, and one of the smaller, *j*, are bisected by the axis of the colony. The remaining four of the larger group may be regarded as forming two pairs, a pair, *i* and *k*, symmetrically placed to the right and left of the axis, nearer the *m* pole of the colony, and nearer together than *o* and *t*, the similarly placed pair toward the *n* pole. The remaining four

smaller spaces also form two pairs, *f*, *p* and *h*, *l*, placed as are the larger pairs but with the nearer pair toward the *n* pole of the colony.

In their relation to the cells between which they occur the intercellular spaces form two classes, those formed at the points where three cells come together and where three walls intersect and those formed by retraction of the plane contact walls of two adjacent cells; in other words, the intercellular spaces which are bounded by three cells and those which are bounded by two cells. There are six of the first class, five in the outer series, and one, *a*, in the inner series, and there are eight of the second class, five in the outer series and three in the inner series.

These spaces, as noted, get their outlines and positions from the tendencies of the cells to assume their hereditary four-lobed form and not merely as an expression of surface tension and rounding up such as results in the triangular intercellular spaces in many loose parenchymatous tissues and in the colony of *Gonium*. The final and definite form of each cell in which it differs from its neighbors arises during growth and in irregular colonies these differences may be very marked (Figs. 25-27). They may consist in inequality in the length of the lobes with greater or less blunting of their tips, curving of the lobes, or deformation of the whole trapezoidal outline of the cell till it becomes rhomboidal or some other form. The growth of the cell will result in the protoplasm as flowage and tensions in the direction of the growing lobes and such tensions exerted at four points of the viscous mass will tend to produce a simple catenoidal deformation of the whole such as arises in all semi-fluid, viscous bodies under tension of any sort. Retraction would naturally occur on the surface of contact midway in the regions of tension simulating a tendency of the whole mass to break up into four droplets. The curves bounding the intercellular spaces in the figures (see Fig. 5 especially) are the obvious expression of tensions exerted on the cell mass in the direction of the four lobes. The degree to which this tendency to catenoidal deformation will come to expression will depend on the viscosity of the protoplasm, the adhesion of the cells to each other, etc. It is in these particulars that the species differ and the same species may under varying conditions or at different stages of development differ in such charac-

teristics. There is no question that *P. Boryanum* may at times, and in the same fashion, develop intercellular spaces, though I have never seen them so symmetrically and strongly developed in this species as in *P. asperum*. The production of the general four-lobed outline is, as evidence given below shows, a result of the inherited form of each cell rather than its pressure relations in the colony.

There is further to be considered a certain suggestion of a want of correlation between the form of the cells and their pressure relations in the colonies of *Pediastrum*. Regarding the young cells as equal and rigid globular bodies in one plane, the central cell in a sixteen-celled group would be in contact with and pressed by five cells symmetrically placed about it. Each of the cells of the second series would be in contact with four cells which are not equally spaced about it. The cells of the third series in such a figure would be alternately in contact with one and two cells, corresponding to the contacts of the cells of series III. in the actual colony which are alternately in contact with three and four cells (Figs. 6, 7). If now the globular cell bodies yield to pressure and flatten upon each other, filling the empty spaces in the group, they will (except the central cell) tend to become oblong and four-cornered like the mature cells of *Pediastrum*. On the other hand, *in the commonest form of the thirty-two-celled colonies the central cell is in contact with the six cells of the second series*, and each of the six assumed globular bodies would be in contact with five cells not similarly placed. In the third series of ten, two such globular cells would be in contact with four each, six would be in contact with three each, and two would be in contact with two each. In the outer series fourteen of the fifteen globular cells would be each in contact with one cell, while one would be in contact with two cells, and yet all these cells in both the sixteen- and thirty-two-celled colonies grow into the characteristic four-lobed form with the resulting contact relations found in the mature colony in which in the sixteen-celled colony, for example, the cells of the second series are each in contact with and pressing against six cells and the cells of the outer series are alternately in contact with one and two cells each. The new specific contacts and pressures arising in growth are due to the inherited four-lobed form of the cells, which none

the less, in my opinion, may have arisen in evolution from the pressure and contact relations of the young cells in the common sixteen-celled colony, regarding them merely as surface tension globules. We may conceive, as is described below, that transitorily the young cells at once flatten upon each other and thus give the figure of *P. Boryanum* with no intercellular spaces. The spaces then start to form with the first growth of the young colony in such a fashion as to make the resulting four-lobed cells as nearly isodiametric as is possible with the numbers involved and the inherited tendency to adhere together in the most compact figure possible (groups of three). It is quite conceivable, then, for the sixteen-celled colony, that out of the incompatibilities resulting from the laws of bipartition and surface tension, both operating in the case of an organism whose cells tend to adhere in colonies, the four-lobed form of the cells has been developed from cell forms such as we find in *P. integrum*. It is easy to recognize certain physical stimuli which have been present; first, surface tension tending to keep each cell isodiametric; second, perhaps during growth, functional hypertrophy as we have observed its action in the case of *Hydrodictyon*; third, adhesion tending to keep the contact surfaces plane; fourth, catenoidal deformation, due to growth tension in the direction of the lobes. Each cell is originally like its fellows—a globular or ovoid bit of jelly. Adhesion during the writhing motions of the final stages of the swarming period leads to its being flattened upon its neighbor. This may be favored by low turgor at this period, but we have little evidence on this point. In *P. Boryanum* the cells, as a rule, adhere permanently over their entire original contact surfaces and thus maintain their primary contact relations. In *P. asperum*, however, the surfaces of the cells tend to separate. The degree and location of this separation may be determined by the tendency of the cells to catenoidal deformation as they grow rapidly in the axes of greatest resistance as developed by their four-lobed form. The cells of the whole colony, with the exception of the central cell, are longer in their tangential axes than in their radial axes, since five cells fill the space which six should occupy in the second series of a least-surface group and ten fill the spaces of twelve in the outer series. Surface tension tends in each cell to equalize these two

axes. Taking cell 4 as an example, the pressures upon it are from the cells 1, 3, 11, 12, 13 and 5 and in tending to round up while developing its pressure relations with these cells it forms the wedge-shaped surfaces of contact inclined to each other at the characteristic angles of 120° . The axes of major growth of the cells after the early stages are just what they would be expected to be on the principle of functional hypertrophy, though as shown below, the cells are capable of reaching their typical form even when their contact with other cells has been reduced to a minimum (Figs. 29-33).

The strongly four-lobed cells are in their form adapted to the expression of a typical surface tension configuration for the whole sixteen-celled colony, but this form does not apparently depend for its expression in ontogeny on the contact relations to which it is so perfectly adapted. Whether in phylogeny, as noted, it was not a result of the cellular interactions existing in the colony is, of course, quite another question. The fluctuating variations in the form of the cells and of the intercellular spaces are the direct expression of the effects of functional response to environmental influences operating in ontogeny. But the cell is able independently to develop its fundamentally four-lobed form.

It may be that we have here a case of an adaptive form character which arose in direct response to and as it were determined by environmental factors of cellular interaction which has become so fixed that it is now transmitted in cell division and comes to typical expression without the need of the stimuli of adhesion and pressure relations which originally called it into being. The considerations here developed apply, of course, especially to the sixteen-celled colony. The case of the thirty-two-celled colony will be considered, as noted below, in another connection.

It is during growth that the intercellular spaces are developed. As the cells of the colony reach their mature size the intercellular spaces become again relatively smaller. The cells now increase their volume by expanding in the direction of least resistance, that is, toward the intercellular spaces. As they prepare for reproduction by swarmspore formation this swelling becomes very marked (Fig. 8). The ovoid spaces become triangular, as does the large

space. The spaces between two cells become flattened to thin bi-convex lense-formed figures. This change of form is accompanied by increased density of the cell content, which also becomes more deeply green in color. It appears that the cells are accumulating reserve storage products to be used in the reproductive period. The distinction between this sort of growth and that at the earliest period, when the cells were developing their long lobes and large intercellular spaces, is obvious and parallels the distinction between the periods of growth and of maturing with the accumulation of reproductive reserves in adult many-celled plants and animals.

REPRODUCTION IN *P. ASPERUM*.

Al. Braun ('51) reported De Bary's discovery of the gametes of *Pediastrum* in 1851 and Askenasy ('88) has described their conjugation and shown the similarity of the life history to that of *Hydrodictyon*. Askenasy ('88) shows fully that the final formation of the new colony is essentially the same process when it comes from a zygospore through the polyeder stage and when it is formed directly by asexual reproduction from a cell of a parent colony.

The method of asexual reproduction in *Pediastrum* has been correctly understood since the work of Meyen ('28), who saw the escape of the swarmspores, their free motion and later their combination to form the young colony and Nägeli ('49) correctly concluded that since the number of cells in a colony is regularly a multiple of two the cells must have arisen by bipartition. To be sure, Conn ('08) rather casually describes the swarmspores as continuing to divide after they have come to rest. Whether this statement of Conn's is based on his own observations or is merely an *a priori* guess at what seems to him probable is not clear. Nägeli ('49) in 1849 had already convinced himself to the contrary. There can be no question but that the process of cell multiplication is completed before the swarming period.

I have photographed more or less successfully a large number of colonies with mother cells at various stages of division. There can be no question that here we have a process of successive bipartition of a multinucleated sporeplasm. Smith ('16) has figured from

sections stages showing the cleavage of numerous segments of the sporeplasm and my photographs show the first division, four-cell stage, etc. Smith's figures show clearly that the cells divide by furrows and that regular karyokinetic figures appear in nuclear division. The swarmspore is uninucleated and the sporeplasm becomes multinucleated before cell division begins.

When the colony is to reproduce itself the cells become extremely plump and to some extent lose their deeply four-lobed form (Fig. 8). The spinous projections tend to be drawn in perhaps, though they do not entirely disappear in any of the species. This is well shown in figures of *P. asperum*, 8, 9, and 10, and at a later stage when cleavage is well along in Figs. 11, 12 and 14. These forms would hardly be recognized as belonging to *P. asperum* if it were not possible to find all conceivable intermediate forms between these very turgid types and the immature colonies with slenderly lobed cells, such as are shown in Fig. 6. In such species as *P. clathratum* (Fig. 3) with its very deep sinuses and slender-branched cells it is plain that the four-pointed cell form maintains itself strongly even against the effects of increase of turgor.

As Askenasy ('88) noted, the nuclei are readily recognizable at this stage. Askenasy mentions, but without figures, that the cell division proceeds by a series of successive bipartitions. Figs. 10, *a* and *b*, show the mother cell dividing into two. Cells *c*, *d*, *e* in the same figure show at least the beginnings of the four-cell stages. It is very difficult owing to the density of the cell contents at these stages to bring out the cleavage clearly in photographs, though the furrows are easily visible on focusing. The divisions apparently occur by constriction furrows from the plasma membrane inward as described by Smith ('16), as noted, and by Timberlake ('02) for *Hydrodictyon*. There is no indication of the formation of cell plates, so far as the appearance of the living material is concerned. That we really have constriction furrows forming here and not merely lines of simultaneous cleavage as claimed by Swingle ('97) for *Stypocaulon* and by various authors for a number of fungi (Baum, '00, Kusano, '09, Barrett, '12, Griggs, '12) is suggested by

the rounding up at the edges of the clefts resulting in open furrows over the surface before the cell is completely cut through.

In these plump-celled forms which are about to reproduce the intercellular spaces are much reduced in area and their form is somewhat changed (Figs. 9-14). The cells may, however, reach maturity and divide without becoming so plump as those shown in the cases just noted. In the stage shown in Fig. 13, cleavage is complete and yet the cells are much more deeply lobed than those in Fig. 11, where cleavage has only reached the eight- to sixteen-celled stages.

The study of the living material is very convincing as to the existence here of division by centripetal furrowing, with rectangular intersection of the cleavage planes. The first cleavage plane is regularly in the short axis of the cell (Fig. 10). The successive planes of division cut each other approximately at right angles through the four- and eight-celled stages. In the sixteen-celled and thirty-two-celled stages there are modifications due to the irregular outline of the mother cells. In general, however, the whole series of divisions tends to illustrate the principle of rectangular intersection.

These processes of division as a rule take place during the night, as Smith has noted ('16), and swarming occurs at daylight, or a little later in cool weather. This is also the case in *Hydrodictyon* ('08). By sealing up with hot paraffine an ordinary water mount containing well-grown colonies of *Pediastrum* this rhythm may be readily disturbed. It is probable that the accumulation of waste products is an important factor in such cases. At any rate in preparations sealed up on the previous evening colonies in various stages of division may be found the next day. The division goes on much more slowly under these conditions or may be inhibited all together. I have kept cultures so sealed for long periods without visible deterioration of the colonies. The young colonies may continue to grow for some time, but after the first day or two no cell division occurs. The colonies may be kept alive for six months if they are kept sealed and not allowed to dry out.

The appearance of the mother cell after division is practically

complete is shown in Figs. 12, 13 and 14. Not all the division lines can be brought out in the photograph. The mass is too thick and it is plain that the swarmspores are in at least two layers. In these reproductive stages, when the cells have become very turgid, and especially during the stages of cell division, the mother colonies tend to lose their flatness and become curved and bent in various ways so that it is extremely difficult to get any large number of cells in focus at once. Colonies with cells in which division is complete when freshly mounted at daybreak are liable at any moment to show the beginning of the swarming period. The cells of a colony rarely swarm all at once. As a rule only part of the cells divide in any one night and in the same way there is a succession in the initiation of active movement in the mother cells, suggesting that internal as well as external factors may be concerned in bringing about the active swarming period. When once the swarm cells begin to move, however, the succeeding stages are normally run through with great speed and with no halting. The daughter cells first seem to round up against each other and lose the rectangular outlines which have been maintained by their mutual pressure. Slight twitchings and quiverings can be seen in the mass whose significance is hard to grasp but almost immediately the swarmers begin to glide upon one another and show writhing, struggling movements. The mother cell now bursts by the familiar crescent-shaped slit on its upper or under surface and its contents slide out in the form of a sack or vesicle containing the writhing mass of young swarmspores. This sack, Al. Braun ('51) rightly observed, is the inner layer of the wall of the mother cell. It is elastic and expands to over twice the diameter of the mother cell as soon as it is free. In thus expanding, however, it still maintains the four-cornered outline of the mother cell, as my photographs (Figs. 18-20) show. The two peripheral spinous projections of the mother cell are still recognizable on it as two symmetrically placed papillæ. In the increased space provided by the expansion of the vesicle the movements of the swarmspores become much more active. The writhings become zigzag dashings to and fro. The swarmspores shoot about in all directions through the mass, which has become much looser, and into the open space

around it. This is a perfectly free swimming movement like that at the corresponding stage in *Hydrodictyon*. Oltmanns ('04, p. 194) remarks that the zoöspores 'probably remain connected by threads of protoplasm but apparently has only Klebs' ('90) mistaken contention as to the corresponding stages in *Hydrodictyon* as the basis for the statement. A single spore can frequently be followed clear through the mass or halfway round its periphery.

This most active swarming period continues for three to four minutes when conditions are favorable. It is followed by a rather sudden slowing down and now the outlines of the future colony appear. The sudden appearance of order out of the chaos of swarming bodies is most striking. The circular outline of the plate appears first and the peripheral cells seem to slow down in their movements, while those in the interior are still quite active. The free-swarming period thus passes over into a second and much longer period of writhing and struggling in which the cells do not move far from their places, but push this way and that between and over each other, crowding and turning around and over without getting completely out of connection with their neighbors as they did in the earlier free-swimming stage. Coincidentally with the slowing down of the movements of the swarmspores they begin to take on the four-lobed form of the adult cells. This change is very conspicuous. The oval swarmspores seem as if they were about to divide into two (Figs. 15-20). Each cell, as the figures show, almost seems to be made up of two pear-shaped halves, the narrowed ends of the halves being the future spines. This sudden assumption by the swarmspores of the four-lobed form is a very striking and conspicuous fact at this stage and is accompanied by rapid growth and mutual pressure between the cells. Walls are formed and the cell contours become more clear cut and definite. All these changes begin with the slowing down of the movements of the swarmspores. The process suggests very strongly the effort of the cells to get into very specific relations with each other and as close together as possible, thus forming the compact plate-shaped colony. As the movement dies away, the behavior of individual cells can be followed with more exactness. A change of position of one cell

apparently may lead to a change of position by all its neighbors and these mutual adjustments and readjustments continue till it seems that a sort of equilibrium is reached. Smith has figured the changes in position of the cells at successive intervals ('16). As noted, the cells in the peripheral series apparently get their definitive positions first, while movement is still quite active in the interior of the young colony. I have not been able to determine just when cilia appear in the swarmspores or when and how they disappear, but doubtless the duration of the active movements of the cells is an index of the limits of the ciliated stage.

To give some hint of the size relations and general appearance of the mother cells and the daughter colonies as they are first formed. I have had reproduced in Figs. 15, 16 and 17 photographs of three stages in the development of a mother cell into a young colony. The difficulties in photographing such stages are very great, as rather long exposures are required and it is not easy to find any considerable number of cells in the same focal plane. Fig. 15 shows the last two cells of a mother colony in which cleavage is complete and swarming is about to begin. All the other cells are already empty and the walls of some of them show faintly. Two young colonies partly out of focus lie nearby. The irregular grouping of the swarmspores in the mother cell shows nothing of the organization of the future colony. There is no evidence of mosaic or any other type of predetermined form inheritance here. In Fig. 16, from a photograph taken a few minutes later, swarming has begun in one of the two mother cells. The vesicle has escaped and lies partly beneath the remaining mother cell. The swarmspores are in active motion and appear only as a gray cloud. It would, of course, require a very short exposure to catch these moving bodies in sharp focus. This figure is less highly magnified than the preceding one and, in all, seven young colonies and part of an eighth are shown lying near, all of them having come earlier from the same parent colony to which the cell just swarming belongs. The figure is printed more deeply, so that the contents of the remaining mother cell appear black, and parts of the outlines of the adjacent empty cells appear more clearly. The attempt was made to focus on the

active mass of swarmspores but the plane of the picture is a little too high and the faintness of the swarming group is partly due to this fact. Fig. 17 is from a plate exposed four or five minutes later. The young colony lies partly beneath the second mother cell but its outlines are in evidence and it is seen to be made up of three concentric rings of cells like the other young colonies lying about. Six of these sister colonies and parts of two more are shown in this figure. The plane of the picture is a little below that of Fig. 16 and other parts of the young colonies are in focus. I have no positive proof of continued ciliary activity in colonies as old as these, but none the less as one watches them they are seen to tip from side to side and even shift their position slightly. Such movements may well be due to slight currents in the water.

As noted, these photographs cannot be regarded as successful, but they give a notion of the relative sizes of the mother cell and the young colony at the time of its birth and the general relations under which the morphogenetic processes take place. With the complete cessation of the movements of its component cells it is obvious at once that the organization of the future colony has been achieved. With the establishment of the peripheral series the young spinous projections are in general all pointing radially outward (Figs. 15-20). In the same way in the inner series, generally speaking, the two-lobed cells have the long axes of their lobes in the radii of the colony viewed as a whole, just as in the full-grown colony. The same exceptions and variations from these rules of orientation can be found in such young colonies as are present in the older ones. The relative number of cells in the outer and inner series, their contacts and the shape of the intercellular spaces are all fixed as they remain throughout subsequent growth and development. Rather rapid growth continues for an hour or so, and with the increase in size of the individual cells their mutual pressure and the development of the intercellular spaces make the specific organization of the colony more conspicuous.

Summarizing, we may distinguish roughly five phases in the vegetative reproduction of *Pediastrum*.

1. Nuclear division which goes on during the vegetative growth of the cells and by which they become multinucleated.

2. Cell division by repeated bipartitions in general according to the principle of rectangular intersections.

3. Escape of the vesicle contemporaneous with quiverings and then slow writhings of the swarmspores for a very brief period, passing at once into

4. The free-swimming stage, lasting several minutes, in which the swarmspores dart about in entire freedom from each other.

5. Slow writhing stage, in which the swarmspores gradually perfect the spatial interrelations found in the adult colony.

The description of reproduction just given may be regarded as representing the process near its optimum as to speed and efficiency. The resulting young colonies (Figs. 15-17) are fairly regular in the arrangement of their cells and rounded in outline. Such conditions are as a rule only achieved in the case of colonies favorably situated and brought under observation only a short time before swarming occurs. In the case of colonies sealed under a cover glass as described above for from six to twenty-four hours before reproduction occurs there are some marked modifications in the process which lead to characteristic changes in the shape of the young colony. One of the commonest deviations from type in the colonies as found in nature is seen in the tendency to be oblong or oval instead of circular in outline. The explanation of this modification in form can be discovered at once by observing the reproduction of colonies which are unfavorably placed. As noted, division and swarming may sometimes be completely inhibited if the colonies are mounted under a cover glass and sealed before they are full grown. If, however, they are about ready to swarm and, for example, are mounted some time in the night before the morning on which they would naturally swarm, the process may take place later in the day. Swarming may thus be delayed for several hours and in such cases it is at once seen that it is less vigorous.

The free-swarming period is shorter or may disappear entirely, the swarmspores only writhing and twisting about without really getting out of contact with each other. The whole active stage is shortened. This may go so far in certain media that the swarmspores scarcely move at all ('88). The fact is to be at once observed in all such cases of a less vigorous and shortened swarming period

that the resulting colonies are never circular in outline but always somewhat elongated in one axis. The spinous projections are less constantly radial in their position and the intercellular contacts and intercellular spaces are far from what I have described above as typical. Photographs of such irregular colonies still inclosed in the mother vesicle are shown in Figs. 18–20. It is difficult, as noted, to bring out the vesicle in a photograph. I have traced over with dilute India ink the outlines of the vesicle in Figs. 18 and 20 to bring out more sharply the points involved. Fig. 19 is left as printed, but the faint outline of the vesicles and their papillæ can be made out. In Fig. 20 an edge view of a young colony in its vesicle is shown. The figures show clearly enough that the long axis of these irregular oblong and oval colonies lies in the long axis of the mother cell. The vesicle, although gelatinous and swollen, is apparently elastic and always maintains the outline of the mother cell even to the retention as noted of two papillæ representing the spinous projections. The conclusion is obvious that these unfavorably situated colonies with reduced activity in swarming are unable to achieve the typical compact circular plate form and the outline of the young colony is influenced by the oblong shape of the enclosing vesicle. The achievement of all the nice adjustments necessary in making a typical least-surface figure requires more effort than the cells of these weakened colonies are capable of and as a result they conform more or less to the outlines of the confining vesicle. Sometimes the result is a flattening of the outline of the colony along one or both edges. Again the result is a more ellipsoidal form. In cases of extreme weakness the colonies may be rather angular (Fig. 23), conforming quite completely to the outlines of the mother cell and, as Askenasy ('88) noted, sometimes not escaping from it. In these extreme cases the colonies are practically always irregular in all their dimensions, with the cells more or less piled upon each other, so that the colony is more than one layer thick. The spinous projections also tend to disappear under these conditions. The effect of environment in modifying and disturbing the morphogenetic processes is thus most clearly shown, and we can class a whole series of such divergences from type as strictly epigenetic and environmental in their origin. The shape and struc-

ture of any given colony of *Pediastrum* conforms to its type in so far as the environment permits. The multiplicity of divergences from the type form for the species is an index of the varying degrees of favorableness in the surroundings of the parent colony and mother cells. The relations of unstable equilibrium between the units of a group of sixteen or thirty-two, as compared with a group of nineteen or thirty-seven, give unusual opportunity for the play of such environmental influences.

FLUCTUATING VARIATIONS IN THE INTERCELLULAR RELATIONS IN THE COLONIES OF *P. ASTERUM* AND *P. BORYANUM*.

The evidence from the above account of asexual reproduction is clear that the colony of *Pediastrum* is formed by the interaction of a group of free-swarming zoöspores without the possibility of any predetermination of its form as such in the arrangement of the parts of the mother cell. A cell can apparently fill any place in the group which forms the young daughter colony. A colony under favorable conditions may attain the rounded outline of a typical least-surface configuration for such a group of cells or under less favorable conditions it may conform more or less wholly to the outline of the mother cell even to the extent of remaining two-layered. I have also described above the general arrangement of the cells and intercellular spaces in a typical adult colony of *P. asperum*, and we may now turn to the question as to the kind and degree of variability which the colonies as they are found in nature exhibit.

In the continuous disk of the typical sixteen-celled colony of *P. Boryanum* I have shown ('16) that the angles of intersection of the cells walls vary considerably in different parts of the colony, the correspondingly placed angles right and left of the axis of the colony tending to be equal. The angles of contact in any fairly typical sixteen-celled colony of *P. asperum* (Fig. 6) are, so far as I am able to determine, quite constantly 120° . The difficulties of measurement here are much greater than in *P. Boryanum*, since the presence of intercellular spaces reduces the surfaces of the cells in contact and the length of the lines by which the angles are measured. Small variations are no doubt quite regularly present, but they are within

the limits of error by any method of measurement I have been able to use. If the ordinary semicircular protractor is used and newly placed for each reading the results show fluctuations of from 1° – 3° between the angles. If, however, the circular protractor with lines marking the angles of 120° is carefully placed so that the three angles can, as it were, be simultaneously read, it is at once apparent how closely the angles approximate 120° , and that the deviations in most cases are so slight as to be practically indistinguishable from appearances due to inequalities in the thickness and density of the cell walls, middle lamellæ, intercellular substances, etc., as shown in the photographs.

TABLE I.

ANGLES OF INTERSECTION OF THE CELL WALLS IN A SELECTED COLONY OF *P. asperum*.

Points.	Angles.		
	<i>a.</i>	<i>b.</i>	<i>c.</i>
g^1	120°	120°	120°
g^2	122	119	119
g^3	120	120	120
g^4	120	120	120
d	120	120	120
d^1	123	120	117
d^2	120	120	120
d^3	120	120	120
d^4	120	120	120
d^5	122	120	118
d^6	121	119	120
d^7	119	120	121
d^8	122	118	120
d^9	120	120	120
Totals.....	1689°	1676°	1675°
Av.....	120°	119°	119°

Higher magnifications making the wall lines bounding the angles longer do not essentially help the situation. I have enlarged to a diameter of about 8 cm. the photograph of *P. asperum* (Fig. 6) and carefully and repeatedly measured the angles at each point of contact for the whole colony. The results are given in Table I. The order in which the angles were read is indicated by the lettering *a*, *b*, *c*, at the points g^1 and *d*. I have not been able in these measurements on *P. asperum* to distinguish between the angles adjacent

to the large and small intercellular spaces, that is, the regions of contact of three and two cells respectively about the points d , d^1 , d^2 , etc., as I did in the case of *P. Boryanum*. Such differences if they are present in *P. asperum* are within the limits of error with the methods of measurement I have so far been able to employ.

The deviations from 120° range from 117° to 123° . The method is not, however, exact and the successive series of measurements from which the averages in the table are derived have little value further than to show plainly enough that the fluctuations here are in most cases relatively slight, and that they appear to range rather equally about 120° for all the cells in the colony.

I have some slight evidence that the inner angle a of the three at the points g^1 , g^2 , etc., and d , d^1 , d^2 , etc., averages a little larger than the other two angles, b and c , but the evidence is by no means adequate on this point and I reserve the data till some further method of testing the questions involved has been devised. It is obvious enough that the fluctuations in the values of these angles of intersection are much less in *P. asperum* than in *P. Boryanum* and this fact is evidently correlated directly with the formation of the large intercellular spaces in *P. asperum*, which permit a curving of the lobes and a general distortion of the whole cell body which results in a more perfect equilibrium between the surface forces at the points of contact of the cells.

The relative degree of conformity to type in a series of the sixteen-celled colonies of *P. asperum* can be better studied by comparing the angles subtended by each cell about the center of the colony. These angles can be measured with more accuracy, and, while they show considerable individual variations, the averages of the rather small numbers obtained show considerable constancy. We may take first a series of fourteen colonies whose cells are arranged in the most common order, namely, one in the center with five around this in the so-called second series and ten in the third or outer series.

It is plain from the table that the bases of the cells of both the second and third series tend to be equal and regarded as arcs of a circle about the center of the colony r (Fig. 7) subtend approximately equal angles. The cells of series II. occupy on the average

TABLE II.

DIMENSIONS OF CELLS IN A SERIES OF FOURTEEN SIXTEEN-CELLED COLONIES OF *P. asperum* AS INDICATED BY THEIR BASES MEASURED AS ARCS OF A CIRCLE ABOUT THE CENTER OF THE COLONY.

Colony.	Cells 2, 3 4, 5 and 6.			
	g^1-g^2 .	g^2-g^3 .	g^3-g^4 .	g^4-g^1 .
294.....	67°	68°	74°	151°
484.....	70	76	68	146
169.....	76	73	74	137
195.....	75	71	74	140
110.....	72	72	76	140
381.....	71	73	71	145
271.....	72	74	65	149
291.....	72	75	70	143
129.....	72	65	73	150
237.....	67	75	73	145
IX.....	76	73	71	140
101.....	72	71	78	139
356.....	66	78	74	142
235.....	74	76	64	146
Totals.....	1003°	1002°	1020°	2013°
Av.....	71°	71°	72°	143°
				71°

	Cells 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16.									
	d^1-d^1 .	d^1-d^2 .	d^2-d^3 .	d^3-d^4 .	d^4-d^5 .	d^5-d^6 .	d^6-d^7 .	d^7-d^8 .	d^8-d^9 .	d^9-d^1 .
294.....	34°	41°	37°	35°	35°	38°	34°	35°	35°	36°
484.....	34	37	36	38	36	34	36	35	37	37
169.....	36	36	36	39	35	36	34	35	34	39
195.....	35	35	39	37	35	36	34	36	32	41
110.....	36	37	36	37	35	35	38	32	36	38
381.....	34	36	35	37	37	36	36	37	39	35
271.....	40	36	36	40	35	36	33	35	34	35
291.....	38	32	38	37	38	37	32	36	36	36
129.....	39	37	39	33	34	34	35	38	34	37
214.....	36	37	32	36	37	35	34	39	37	37
107.....	33	40	30	38	37	40	38	36	35	33
IX.....	32	38	34	39	36	37	37	37	36	33
101.....	37	34	36	40	36	36	35	35	36	35
356.....	37	35	38	35	40	37	36	35	33	34
235.....	34	32	40	38	39	32	37	38	40	30
Totals....	535°	543°	542°	559°	545°	539°	529°	539°	534°	536°
Av.....	35.6°	36.5°	36.1°	37.1°	36.3°	35.9°	35.2°	35.9°	35.6°	35.7°

from $71^\circ +$ to $72^\circ +$, 72° being the typical angle for such a group. I have measured the angles occupied by cells 2 and 6, together, as the arc g^1-g^4 which subtends in the average of the fourteen colonies an angle of 143° . Dividing this equally between cells 2 and 6 gives

them each an angle of $71^\circ +$. The smallest angle for any of the cells in series II. in all the fourteen colonies is 64° in colony 235, the largest is 78° in colonies 101 and 356. The range of fluctuating variation is hence in the fourteen colonies 14° or a little less than 20 per cent. of the normal.

The bases of the cells of series III., as the table shows, subtend angles varying in the averages from 35.6° to 37.1° , 36° being the typical angle. The smallest angle in this series is 32° and the largest 41° , a range of fluctuation of 9° . While the number of colonies whose angles have been measured is not large, it seems to me evident from the data that there is a marked tendency to equality in the spaces occupied by the cells in each series and that the typical configurations about which fluctuation occurs would give each of the cells of series II. 72° and each of the cells of series III. 36° . If we arrange the angular measurements of the cells in series II. in a series so as to show the frequency of angles of each particular value, while there are marked irregularities in the series, we have evidence of chance distribution about the value 72° as a mode, 31 angles measuring more than 72° and 29 angles measuring less than 72° (see Table III.). Treating the angles of series III. in the same way we find in spite of fluctuating irregularities 59 angles below the mode at 36° and 58 angles above the mode (Table III.). Here again there is evidence of chance distribution about 36° , the value of the typical angle for such a group with least-surface configuration.

TABLE III.

FREQUENCY OF THE ANGLES OF THE VARIOUS VALUES.

Cells 2-6 Inclusive.

64°	65°	66°	67°	68°	69°	70°	71°	72°	73°	74°	75°	76°	77°	78°
1	2	1	2	4	2	8	9	10	9	8	7	5	0	2

Cells 7-16 Inclusive.

30°	31°	32°	33°	34°	35°	36°	37°	38°	39°	40°	41°
2	0	8	6	17	26	32	25	14	9	8	2

For *P. Boryanum* I have elsewhere ('16) given the data as to the organization of the colony based on the study of an individual selected for its regularity. The comparison of the type dimensions obtained in this fashion with those obtained from averaging a series

TABLE IV.

VALUES OF THE INCLUDED ANGLES OF THE CELLS OF A SERIES OF SEVEN SIXTEEN-CELLLED COLONIES OF *P. Boryanum* ARRANGED IN THE TABLE AS THEY WERE MEASURED ABOUT THE POINTS g, g^1, g^2 , etc., e, e^1, e^2 , etc., and d, d^1, d^2 , etc.

Colony.	Angles about Point g .		
	i^2gi .	i^2gk .	igk .
87.....	114°	116°	130°
131.....	115	120	125
1-d.....	122	113	125
1-b.....	108	124	128
39.....	120	120	120
23.....	115	117	128
55.....	111	121	128
Totals.....	805°	831°	884°
Av.....	115°	117°	126°
		Totals.....	117
		Av.....	243°
			121°

Colony.	Angles about Point g^1 .			Angles about Point g^4 .		
	i^1g^1i .	k^1g^1i .	$k^1g^1i^1$.	$i^4g^4i^4$.	$i^4g^4k^4$.	$i^4g^4k^4$.
87.....	108°	121°	131°	112°	117°	131°
131.....	100	119	141	107	117	136
1-d.....	108	125	127	96	132	136
1-b.....	115	112	133	107	126	127
39.....	102	136	122	105	120	135
23.....	105	119	136	112	121	127
55.....	113	125	121	102	129	129
Totals.....	751°	857°	911°	741°	862°	921°
Av. right.....	107.2°	122.4°	130.1°	105°	123°	131°
Av. left.....	105.8	131.5	123.1			
Totals.....	213°	253.9°	253.2°			
Av.....	106.5°	126.9°	126.6°			

Colony.	Angles about Point g^2 .			Angles about Point g^4 .		
	$i^2g^2i^2$.	$i^2g^2k^2$.	$i^2g^2k^2$.	$i^4g^4i^4$.	$i^4g^4k^4$.	$i^4g^4k^4$.
87.....	101°	133°	126°	77°	149°	134°
131.....	91	130	139	88	133	139
1-d.....	92	140	128	90	138	132
1-b.....	93	138	129	99	131	130
39.....	86	141	133	91	138	131
23.....	89	136	135	98	135	127
55.....	81	136	142	93	134	132
Totals.....	633°	954°	932°	636°	958°	925°
Av. right.....	90.3°	136.2°	133.1°	90.8°	136.8°	132.1°
Av. left.....	90.8	132.1	136.8			
Totals.....	181.1°	268.3°	269.9°			
Av.....	90.5°	134.1°	134.9°			

TABLE IV.—*Continued.*

Colony.	Angles about e .		
	oeo° .	$oe k^{\circ}$.	keo° .
87.....	152°	96°	112°
131.....	134	120	106
1-d.....	131	105	124
1-b.....	—	—	—
39.....	133	112	105
23.....	129	111	120
55.....	137	108	115
Totals.....	816°	652°	682°
Av.....	136°	108°	113°

Colony.	Angles about e^1 .			Angles about e^2 .		
	$o^1e^1o^2$.	$k^1e^1o^2$.	$k^1e^1k^2$.	$o^1e^1o^2$.	$k^1e^1o^2$.	$k^1e^1k^2$.
87.....	139°	109°	120°	130°	105°	125°
131.....	146	97	117	136	108	116
1-d.....	132	112	116	120	120	120
1-b.....	—	—	—	—	—	—
39.....	143	113	104	146	111	103
23.....	137	107	116	139	107	114
55.....	132	109	119	145	106	109
Totals.....	829°	647°	692°	816°	657°	687°
Av.....	138°	107°	115°	136°	109°	114°

Colony.	Angles about e^1 .			Angles about e^2 .		
	$o^2e^2o^1$.	$o^2e^2k^1$.	$o^2e^2k^2$.	$o^2e^2o^1$.	$k^2e^2o^1$.	$k^2e^2k^2$.
87.....	125°	120°	115°	126°	121°	113°
131.....	129	112	119	136	112	112
1-d.....	—	—	—	126	114	120
1-b.....	—	—	—	—	—	—
39.....	140	100	120	130	110	120
23.....	143	103	114	128	114	118
55.....	149	95	116	132	108	120
Totals.....	686°	530°	584°	778°	679°	703°
Average.....	137°	106°	116°	129°	113°	117°
			e^2	137	106	116
			e^3	138	107	115
			e^4	135	109	113
			e^1	136	109	114
			Totals...	675°	544°	575°
			Av.....	135°	108°	115°

TABLE IV.—Continued.

Colony.	Angles about d^b .			Angles about d .		
	$d^b d^b p^b$.	$r^b d^b p^b$.	$r^b d^b o^b$.	odp .	rdp .	rdo .
87.....	120°	120°	120°	120°	120°	120°
131.....	105	127	128	109	123	128
1-d.....	99	127	134	118	120	122
1-b.....	—	—	—	—	—	—
39.....	116	116	128	118	115	127
23.....	102	118	139	119	120	121
55.....	109	129	122	109	126	125
Totals...	651°	737°	771°	693°	724°	743°
Av.....	108.5°	122.8°	128.5°	Right.....115.5° Left.....108.5°	120.6° 122.8°	123.8° 128.5°
				Totals.....224°	243.4°	252.3°
				Av.....112°	121.7°	126.1°

Colony.	Angles about d^b .			Angles about d^1 .		
	$o^b d^b p^b$.	$r^b d^b p^b$.	$r^b d^b o^b$.	$p^1 d^1 o^1$.	$r^1 d^1 p^1$.	$r^1 d^1 o^1$.
87.....	108°	123°	129°	—°	—°	—°
131.....	114	120	126	105	126	129
1-d.....	98	132	130	106	126	128
1-b.....	—	—	—	—	—	—
39.....	116	118	126	120	120	120
23.....	120	113	127	116	120	124
55.....	107	128	125	113	120	127
Totals...	663°	734°	763°	560°	612°	628°
Av.....	110.5°	122.3°	127.1°	Right.....112° Left.....110.5°	122.4° 122.3°	125.6° 127.1°
				Totals.....222°	244.7°	252.7°
				Av.....111°	122°	126°

Colony.	Angles about d^1 .			Angles about d^2 .		
	$o^1 d^1 p^1$.	$r^1 d^1 p^1$.	$r^1 d^1 o^1$.	$o^2 d^2 p^2$.	$r^2 d^2 p^2$.	$r^2 d^2 o^2$.
87.....	111°	120°	129°	102°	131°	127°
131.....	111	122	127	103	127	130
1-d.....	—	—	—	87	131	142
1-b.....	—	—	—	—	—	—
39.....	116	124	120	117	128	115
23.....	—	—	—	100	127	133
55.....	97	129	134	112	124	124
Totals...	435°	495°	510°	621°	768°	771°
Av.....	108.7°	123.7°	127.5°	Right.....103° Left.....108	128° 123	129° 127
				Totals.....211°	251°	253°
				Av.....105°	125°	127°

TABLE IV.—Continued.

Colony.	Angles about d^2 .			Angles about d^3 .		
	$d^2d^3d^4$.	$r^2d^3d^4$.	$r^2d^3d^2$.	$d^3d^2d^4$.	$r^2d^3d^2$.	$r^2d^3d^4$.
87.....	110°	122°	128°	90°	139°	131°
131.....	90	128	142	105	125	130
1- d	—	—	—	100	131	129
1- b	—	—	—	—	—	—
39.....	112	127	121	101	123	136
23.....	—	—	—	—	—	—
55.....	112	125	123	104	123	133
Totals ...	424°	502°	514°	496°	641°	659°
Av.....	106°	125.5°	128.5°	Right.....100°	128°	131°
				Left.....106	125	128
				Totals.....206°	253°	259°
				Av.....103°	126°	129°

Colony.	Angles about d^3 .			Angles about d^4 .		
	$d^3d^2d^4$.	$r^2d^3d^4$.	$r^2d^3d^2$.	$d^4d^3d^2$.	$r^2d^3d^4$.	$r^2d^3d^4$.
87.....	105°	129°	126°	100°	131°	129°
131.....	114	126	120	105	128	127
1- d	—	—	—	108	126	126
1- b	—	—	—	—	—	—
39.....	111	117	132	104	127	129
23.....	—	—	—	—	—	—
55.....	114	120	126	108	129	123
Totals ...	144°	492°	504°	525°	641°	634°
Av.....	111°	123°	126°	Right.....105°	128°	126°
				Left.....111	123	126
				Totals.....216°	251°	252°
				Av.....108°	125°	126°

of selected individuals is made possible with the data given below in Table IV. The lettering of the angles, etc., as noted above, is given in Fig. 35 which is taken from my earlier paper ('16, Fig. 1b).

The comparison of the values of the included angles made by the intersection of the cell walls in two such types as *P. Boryanum* and *P. asperum* brings out very clearly the significance of the cell form as related to the symmetry of such groups. As noted in *P. Boryanum* the angles fluctuate widely, while in *P. asperum* their variations are almost entirely within the limits of error in measuring them.

The data given in Table IV. are based on measurements of seven colonies of *P. Boryanum*, all of which again have the common arrangement of their cells, that is, one cell in the center, five in the second series, and ten in the third series with the long lobes of the cells of series II. and III. directed radially outward. The lettering of the angles, etc.; is given as noted in the type diagram. These seven individuals are all relatively regular and symmetrical and the angles of intersection of their cell walls do not fluctuate so widely as is the case in colonies whose cells are more irregularly placed (Figs. 26-28). It seems, however, impossible to include these more irregular colonies with the more symmetrical types in a comparison of the values of the included angles of their cells, since, as I have pointed out already, the typical colonies tend to be bilaterally symmetrical and a comparison of fluctuation in the included angles of their cells can only be of significance if made between correspondingly placed angles (homologous angles of Rhumbler ('02)). In the irregularly arranged colonies it is quite impossible to compare angles as similarly placed with reference to the pressure relations in the colony. In the case of these irregular colonies I have brought together the values of the included angles of all their cells and classified them according to their value in degrees without reference to their specific positions in the colony. The data so obtained will be briefly discussed later. The seven colonies whose angles are here compared are a selected group and the figure which is obtained by averaging their values we may call for convenience the diagram of the average of the common type. I have not drawn this figure as a larger number of measurements is needed to give such a drawing fundamental significance. The reëntering angles of the cells and our lack of quantitative knowledge of the adhesion and viscosity of the protoplasm make it impossible to compute a theoretical type configuration of sixteen units in such a group which might be made a standard of comparison. The figure derived from averaging the corresponding elements in an exceptionally regular individual as given in my earlier paper we may similarly call the type diagram from a selected individual. The significance of the measurements of such a series of fairly regular colonies may be best discussed in connection with the measurements of the single colony selected for its symmetry.

In Table V. I have brought together the average values of each of the included angles of the cells arranged as right and left pairs and the averages of these pairs in comparison with the corresponding measurements of the selected colony No. 55 which I gave as noted in a former paper ('16, pp. 98, 99).

The table, section I, gives in order the values of each of the five included angles of the central cell and of the included angles of the cells of series II. and III. in these seven colonies arranged in right and left pairs and averaged. In the right-hand column are the corresponding values for the selected colony, No. 55. For convenience we may call the unpaired angle of the central cell, igi^6 , the basal angle. The right and left pair of angles ig^1i^1 and $i^5g^4i^6$ the lateral angles, and the remaining pair which measure the two lobes of the cell ig^2i^3 and $i^4g^3i^5$ the apical angles. In the cells of series II. (except cell 4) we shall have two basal, two lateral and two apical angles and in cell 4 and in all the cells of series III. three basal angles, for example, in cell 12 $p^4d^4r^4$, $p^5d^5r^5$, and $p^4h^3p^5$ and in cell 4 the corresponding three angles.

In the central cell the unpaired angle igi^6 on the axis of the colony is the largest of the five in both cases. It is 115° for the average of the series and 111° for the single colony. The apical pair of angles bounding the tips of the lobes $i^2g^2i^3$ 90° and $i^4g^3i^5$ 90° are the smallest of the five. Their values are nearly equal in the averages from the series, as they should be for the bilateral symmetry of the colony. In the single colony, No. 55, they are markedly unequal, 81° and 93° , though their average, 87° , is only three degrees different from the average of the series 90° .

The average values of the lateral angles ig^1i^1 107° and ig^5i^6 105° are intermediate between those of the basal and apical angles of the cell in the averages of the series as they also are in the single colony. In the latter again they differ by 11° though their average, 108° , differs by only 2° from that of the series, 106° . On the whole, the differences between the typical shape of the central cell as determined from the selected individual and as determined by the average of a selected series are within the range of errors in measurement by the methods used for angular dimensions in photographs of such objects.

TABLE V.

AVERAGE VALUES OF EACH OF THE INCLUDED ANGLES OF THE CELLS AND AVERAGES OF THE RIGHT AND LEFT PAIRED ANGLES IN A SERIES OF SEVEN 16-CELLED COLONIES OF *P. Boryanum* COMPARED WITH THE CORRESPONDING ANGLES IN THE SELECTED TYPE DIAGRAM. ('16 pp. 98, 99.)

1. Includes Angles of the Central Cell.

				Average.	Type diagram.
i^6gi	115°			115°	111°
$i^1g^1i^1$	107°	$i^6g^4i^6$	105°	106°	108°
$i^1g^2i^3$	90°	$i^4g^2i^5$	90°	90°	87°

2. Basal Angles of Cells 2-6.

igk	126°	i^6gk	117°	121°	124.5° 126° 134° 138°
ig^1k^1	122°	$l^6g^4k^4$	131°	126°	
$i^1g^1k^1$	130°	$i^6g^4k^4$	123°	126°	
$i^1g^2k^2$	136°	$i^6g^4k^4$	132°	134°	
$i^3g^2k^2$	133°	$i^4g^2k^2$	136°	134°	
Totals.....	647°		639°	641°	
Av.....	129°		127°	128°	

3. Lateral Angles of Cells 2-6.

keo	108°	keo^9	113°	110°	111° 107° 120°
$k^1e^1o^1$	114	$k^4e^4o^5$	107°	110°	
$k^1e^1o^2$	109	$k^4e^4o^7$	115°	112°	
$k^2e^2o^3$	117	$k^2e^2o^6$	106°	111°	
$k^2e^2o^4$	113	$k^3e^3o^5$	116°	114°	
Totals.....				557°	338°
Av.....				111°	112°

4. Apical Angles of Cells 2-6.

odp	115.5°	$o^9d^9p^9$	108.5°	112°	108° 108°
$o^1d^1p^1$	112.0	$o^6d^6p^6$	110.5	111.2	
$o^2d^2p^2$	103.0	$o^7d^7p^7$	108.7	105.8	
$o^3d^3p^3$	100.0	$o^8d^8p^8$	106.0	103	
$o^4d^4p^4$	105.0	$o^6d^6p^6$	111.0	108	
Totals.....	535.5°		544.7°	540.0°	
Av.....	107.1°		108.9°	108°	

5. Mid-basal Angles of Cells 7, 9, 11, 13 and 15.

oeo^8	136°			136°	137° 133°
$o^1e^1o^2$	136	$o^7e^4o^8$	138°	137	
$o^2e^2o^4$	129	$o^6e^2o^6$	137°	133	

TABLE V.—Continued.

6. Basal Angles of Cells 7-16.

pdr	120°	$p^3d^3r^9$	122°
$p^1d^1r^1$	122	$p^3d^3r^8$	122
$p^2d^2r^2$	128	$p^7d^7r^7$	123
$p^3d^3r^3$	128	$p^6d^6r^6$	125
$p^4d^4r^4$	128	$p^5d^5r^5$	123
Totals	626°		615°
Av.	125°		123°
odr	123°	$o^3d^3r^9$	128
$o^1d^1r^1$	125	$o^3d^3r^8$	127
$o^2d^2r^2$	129	$o^7d^7r^7$	127
$o^3d^3r^3$	131	$o^6d^6r^6$	128
$o^4d^4r^4$	126	$o^5d^5r^5$	126
Totals	634°		636°
Av.	126°		127°

The basal angles of the cells of series II. igk , i^6gk , ig^1k^1 , $i^6g^4k^4$, etc., also constitute five pairs of correspondingly placed angles right and left of the axis of the colony mn . The average values of each for the series of seven colonies are arranged in corresponding pairs in the table, section 2. In this series the average values of the right and left pairs are seen to be progressively smaller as we pass in the direction from pole n towards pole m in the colony. The values range from 121° in the first pair about the point g to 134° in the pairs about g^2 and g^3 . The equivalence of the adjacent basal angles of cells 2-3, 126°, and 3-4, 134°, is to be expected as a characteristic of the type configuration though it is more or less accidental doubtless that it should appear on the basis of so few measurements. The progressive increase in size of the basal angles of these cells is correlated with the reduction in value of the corresponding included angles of cell 1 proceeding from basal to lateral and apical angles. As shown in the table, column 4, the values of the corresponding angles in the selected type diagram from colony 55 agree fairly well with these averages from the seven colonies. The first pair are smaller in the average from the series. The next three pairs are the same, and the last pair, the basal angles of cell 4, are smaller in the average type than in the selected type.

We have two sets of points of intersection between the walls of the cells of series II. and series III., due to the fact that there are ten cells in series III. to five cells in series II. We may consider first the included angles of cells 2-6 about the five points of intersection marked ee^1e^2 , etc. The values of these angles for the seven colonies and their averages are given in Table IV. Point e is on the axis. The remaining four points, e^1e^4 , are placed symmetrically right and left of the axis nm as is indicated by their position in the table. The average values of the lateral angles of cells 2, 3, 4, 5, and 6 about these points e , e^1 , etc., are arranged in five corresponding right and left pairs in Table V., section 3.

There is no adequate evidence of any regularly progressive change in value in the averages of these five pairs as compared with the progressive change in value of the basal angles of these cells. This is doubtless owing to the increased distance of these angles from the five-sided central cell with its unequal included angles, due to its inherited form tendencies. There is a manifest tendency for the angles of intersection of the cell walls to become equal in harmony with the general symmetry of a least-surface configuration. The values of these angles from the averages of the series agree fairly well with the values of the corresponding angles in the selected type figure. In the case of the lateral angles of cell 4, an arbitrary value, 120° , was assigned in the diagram. The actual average value of these angles in colony 55 was 112° , as shown in the table ('16, p. 98), and this is only two degrees from the average value of these angles in the series of seven colonies.

The values of the right and left pairs of the apical angles of cells 2-6 are given in Table V., section 4. There is considerable fluctuation in the values of these angles but the average of the series, 108° , agrees closely with the value obtained for the same angles in the selected colony 55. In this case again an arbitrary value (100°) was given to the apical angles of cell 4 in the type diagram and the average for the series agrees with the measurements from the selected colony, 109° , and not with the arbitrarily assigned value.

There is no clear evidence from the series that there is any progressive change in value of these apical angles odp , $o^1d^1p^1$ of the

cells of series II. as we pass from pole *n* to pole *m* of the colony. The average values of these angles, comparing all seven colonies, have an extreme range of 9° from 103° to 112° , with, as noted, an average of the averages for all the colonies of 108° . In the single colony, No. 55, the range of variation was 17° from 97° to 114° . Colony No. 87 showed a range of variation of 30° from 90° to 120° in the values of the angles odp , $o^1d^1p^1$, etc.

The average values of the five mid-basal angles of cells 7, 9, 11, 13 and 15 are given in Table V., section 5. We have here a case in which the average values of the angles in the series of seven colonies is the same as the value (133°) arbitrarily assigned in the selected type diagram for the mid-basal angles of cells 11 and 13, and differs by seven degrees from the average value as measured (140°) of these two angles in colony 55, from which the selected type diagram was derived. This is the reverse of the result as we have found it in the other two cases noted above and a fourth case noted below in which an arbitrary value was given to angles in the selected type diagram. In the three other cases the average value of the angles in colony 55 as measured is nearer to the average value of the corresponding angles in the series of seven colonies than to the arbitrarily assigned value. As I have already pointed out ('16), the angles about the point e^3 in colony 55 are obviously to the eye the most unsymmetrical and aberrant in the whole colony and the possibility of making consistent arbitrary corrections for these asymmetries and their correlated effects on the other angles in the region is not very great. The values of the unpaired angle $o eo^9$ and the pair at e^1 and e^4 agree closely with those obtained from the selected colony No. 55 and given in the selected type diagram. The values of these mid-basal angles of the peripheral cells are larger than those of the other angles of the groups about the points of intersection e , e^1 , e^2 , etc., and this we may regard as a direct correlative of the fact that the tangential diameters of the cells of series II. and III. are regularly greater than their radial diameters. If this tangential elongation of the cells were directly determined by the fact that there are but five cells in series II. and ten cells in series III., instead of six and twelve, the normal numbers for a least-surface configuration in which all three angles would be 120°

each, we might be able to give a fixed value to this relation between the inequality of the diameters and the inequality of the angles about e and to get some notion of the viscosity of the protoplasm, the adhesion of the cells to each other, etc. *As noted, however, it is obvious that the inequality of the diameter may exist in some degree at least in approximately free cells, as is shown in figures 29-33.*

In the basal included angles of cells 7, 8, 9, 10, etc., of series III., we have two series consisting of five pairs each placed symmetrically right and left of the axis mn of the colony, as in the case of the corresponding angles, about gg^1 , etc., and ee^1 , etc. The value of each of these angles for each of the seven colonies is given in Table IV. The averages arranged as two sets of corresponding right and left pairs are given in Table V., section 6. The agreement in the values of the averages of these sets is rather close, the range being from 123° , the smallest, to 127° , the largest. It seems probable that in the type configuration determined by surface tension and the inherited shape of the cells these angles tend to be equal. The average for the different sets is:

For the Series.	Type Diagram.	Colony No. 55.
125°	127°	127°
123°	123°	123°
126°	130°	130°
127°	131°	123°
Total..... $\underline{501^\circ}$	128°	128°
Av. 125°	$\underline{635^\circ}$	$\underline{631^\circ}$
	127°	126°

In the selected type diagram derived from the selected colony, No. 55, the average for these angles is 127° . The colony No. 55 is, however, especially irregular in the region of cell four, and an arbitrary value, 131° , was assigned to the two angles $o^4d^4r^4$ and $o^5d^5r^5$. If the measured value for these two angles, 123° , is taken instead of the arbitrary value, the average of these angles for the single selected colony, No. 55, becomes 126° , only 1° different from that for the series.

On the whole, the values for the included angles of the cells agree fairly well when derived by averaging the corresponding

pairs in a series of colonies of similar cell arrangement with those derived by averaging the values of the corresponding pairs of angles in a colony selected for its obvious symmetry.

I have emphasized the difficulty of measuring the angles in photographs of such small objects with the lack of sharpness in the cell walls, especially at points of intersections, etc. Only approximations can be achieved with the technique I have used. The fact that the colonies are not absolutely flat figures so that the plane of the photograph cuts the colony at different levels and the further fact that the walls are not absolutely vertical to the plane of the colony present difficulties inherent in the nature of the material.

The average values of the angles measured from the center of the colony which the corresponding cells in the series of seven colonies subtend are given in Table VI. The exact determination of the center of the colony is not easy in view of the irregularities of its boundaries. The point taken as the center in each colony was midway between the point of intersection of the major axes of the colony and the point of intersection of the major axes of the central cell. The values obtained from averaging the series like those for the single selected colony No. 55 and those of the corresponding angles in *P. asperum* (Table II.) are fairly close to 36° , the angle for a strictly surface tension configuration. There can be little doubt that these angles tend to be equal as would be expected were surface tension and adhesion alone operative.

TABLE VI.

DIMENSIONS OF CELLS 7-16 IN A SERIES OF SEVEN COLONIES OF *P. Boryanum*
AS INDICATED BY THEIR BASES MEASURED AS ARCS OF A CIRCLE
ABOUT THE CENTER OF THE COLONY.

Colony.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
87.....	38°	30°	40°	35°	37°	30°	38°	36°	35°	41°
131.....	37	35	35	36	36	36	36	36	37	36
1a.....	35	37	35	37	36	38	34	34	37	37
1b.....	—	—	—	—	38	34	38	33	36	38
34.....	35	35	39	32	37	40	36	35	36	35
23.....	37	37	37	32	41	34	41	28	40	33
55.....	35	36	34	36	36	38	36	36	35	38
Totals....	217°	210°	220°	208°	261°	250°	259°	238°	256°	258°
Av.....	36°	35°	36°	34°	37°	35°	37°	34°	36°	36°

How much significance can be attached to the degree of agreement obtained by the two methods used in obtaining the norms of all these fluctuating elements can only be determined by more extensive statistical studies both of especially symmetrical single colonies and extensive series of colonies chosen only on the basis of their having a similar arrangement of their cells. The tendency to equality shown in the values of the corresponding angles both for the intersection of the walls and for the spaces occupied by the entire cells certainly suggests that the structure of the colony tends to be a least-surface configuration with all angles of intersection of the cell walls equal to 120° and all the cells subtending equal angles about the center of the colony, this tendency being in every case limited, however, by the inherited form of the cells and the accidents of environment.

The results of my measurements of the homologous angles in the colonies of *Pediastrum* are in agreement with Rhumbler's ('02) results obtained by measuring the homologous marginal angles of various Foraminifera and confirm still further the conception of the semi-liquid nature of plant and animal protoplasm. Rhumbler uses these results primarily as evidence on this point. The fact that only homologous angles tend to be equal leads him at once, however, to emphasize the heterogeneity in structure of the protoplasm resulting in an anomogenous consistency and anomogenous tensions in different regions of the cell. The cell of *Pediastrum* with its inherited four-lobed form operating always with surface tension in determining the value of any given cell angle is also a notably anomogenous system, as compared with a simple fluid droplet. In the morphogenesis of the colony it is obvious that this anomogeneity is quite as important a factor as is the principle of surface tension. It is in the indisputable evidence from both Rhumbler's material and my own of the interplay of capillarity, protoplasmic anomogeneity, and especially of the principle of binary fission that we get a basis for interpreting the complexity of the form elements with which we are confronted even in such simple organisms as the Foraminifera and cœnobic algæ. The least-surface configuration comes to expression in *Pediastrum* in so far as is consistent with the inherited cell form and consistency and with

cell numbers produced by bipartition. The endless variations in form found on comparing a series of colonies are the expression of the unstable equilibrium, arising especially from the simultaneous operation of the law of bipartition and the physical principle of least surfaces.

In the series of colonies chosen I have included as noted only individuals with the common plan of $1 + 5 + 10$, since it is impossible to compare the angles fairly in individuals with different geometric plans. This is obvious in the case of the superficially

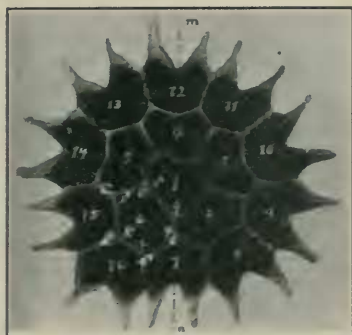


FIG. 25. *Pediatrum Boryanum*. Colony with cell number 6 so displaced that the side which should be adjacent to cell 5 is in contact with cell 1, the side that should be in contact with cell 1 is in contact with cell 2, etc. The remainder of the cells are normally placed. $\times$ about 600.

rather regular colony numbered 67 (Fig. 25). In this colony all the cells are regularly and symmetrically placed except No. 6, in series II. This cell has swung around until its major axes, both radial and tangential, are displaced about 45° . Its reëntering angle normally under the middle of cell 16 is now under cell 15. The entire cell has been, broadly speaking, rotated through one sixth of its circumference so that each of its sides has been displaced by one in its relations with the sides of the neighboring cells. The side normally adjacent to cell 5 is now adjacent to cell 1. The side normally adjacent to cell 1 is now adjacent to cell 2 and much reduced in length. The side normally adjacent to cell 2 is now adjacent to cell 7, etc. Cells 15 and 7 have slipped in toward the center of the colony so that its peripheral outline has been flattened

at these points. The central cell is more nearly isodiametric, etc. In such a colony it is plain that the included angles of cell 6, and those of all its immediate neighbors at least, are under quite different pressure relations than they would be with the normal cell arrangement. Their fluctuations will be of a different order than if they were normally placed. The writhing, struggling motions of the final stage of swarming as described above could only have resulted in the normal relations of equilibrium in case they had been of sufficient violence to bring the cell out of its present abnormal relations into the normal so that the properly matched sides of it and its neighbors would be in contact.

The cell has reached a condition of equilibrium in its pressure relations with the adjacent cells, but this equilibrium has not achieved anything like equivalence in value or position for the corresponding sides and angles. The palpable asymmetry in this cell 6 and its neighbors is most convincing proof that the form of the cell is not influenced merely by its pressure relations in the colony, but also by its inherited tendency to assume the characteristic four-lobed outline. The displacement of cell 6 is brought out very clearly by comparing the right and left pair of cells 3 and 5 with the right and left pair 2 and 6. In spite of fluctuating variations cell 3 could be superimposed on cell 5 by merely rotating it on the axis of the colony *mn* through an angle of 180° . Whereas to superimpose cell 2 on cell 6 there would be necessary a further rotation of No. 6 about its center and in the plane of the colony through about 45° so as to bring the corresponding sides and angles of the two cells together.

Colony 67 illustrates asymmetry originating in the displacement of a single cell to the extent of bringing unmatched sides together. This we may call anomogenous asymmetry as contrasted with the asymmetries involving merely fluctuation in the values of sides and angles without the passing of a critical point which alters the fundamental arrangement of the cells by bringing about abnormal juxtapositions of sides and angles.

All grades and degrees of this anomogenous displacement of cells can be found in nature. Two general types of irregularity can be distinguished. The first is an irregularity which does not pri-

marily affect the outline of the colony or the arrangement of the cells in concentric series about a center, the displacement being largely in the position of the major axes of the individual cells. This is illustrated as noted in colony 67 (Fig. 25). The second results in an abnormal form for the colony as a whole and the loss of the concentric arrangement of the cells (Figs. 26 and 28). The two types are more or less combined, of course, in the majority of cases.



FIG. 26. *Pediatrum asperum*. Colony irregular, crescent-shaped form. $\times$ about 425.

FIG. 27. *P. asperum*. Colony fairly regular in outline but interior cells very irregularly placed. $\times$ about 275.

FIG. 28. *P. asperum*. Irregular colony, somewhat triangular in outline. $\times$ about 425.

The most extreme case of the first type which I have observed is seen in the thirty-two-celled colony (Fig. 27). Here almost all the interior cells are anomogenous in their position and interrelations with their neighbor cells, though there is the common general arrangement for a thirty-two-celled colony; one in the center, six cells in series II., ten cells in series III., and fifteen cells in series IV. All the cells of the outer series are normally placed with long spines outward and the general outline of the colony is circular, but the interior cells are in absolute confusion, as compared with the typical arrangement. Only three cells of series III. have their long lobes radially outward and their long axes tangential and these three are not in normal contact relations with the cells of series II. This colony emphasizes the existence of the stage described under the head of reproduction, where it was noted that the cells of the peripheral series seem to get their definitive positions first while the

interior cells are still actively swarming. The appearance here is as if the development of the colony had proceeded normally until the peripheral cells had gotten their places and then for some reason activity was checked before the interrelations of the cells in the interior had been worked out. It would be possible to express all this confusion in degrees of displacement of the major axis of each cell, but the situation is sufficiently clear from a comparison of this colony with that shown in Fig. 4 without giving it such a mathematical expression. I shall include data on this point in a later study from a wider comparative viewpoint involving the general effect of increasing numbers of cells on the organization of the colonies.

An extreme of irregularity of the second type which is expressed in the general contour of the colony is shown, as noted above, in Fig. 26. Here the colony is crescentic in outline. The type arrangement of cells for a sixteen-celled colony has entirely disappeared and we can no longer recognize a central cell with two concentric series about it and yet the inherited form of the cells is quite perfectly developed in all cases. The modifications are such as are obviously due to the special pressure relations under which each cell finds itself. It is possible, of course, in many cases to identify all the angles of any particular cell and to combine them in homologous groups for the colony and a whole series of colonies, as I have done for the series of more regular individuals. But in these irregular colonies the major axes of the colony are frequently quite unrecognizable and any particular included angle of a cell will be so obviously misplaced and with such unusual pressure relations that the comparison of its values in different colonies becomes a very complex problem.

I have brought together in Table VII. the values of all the included angles in a series of both regular and irregular sixteen-celled specimens of *P. Boryanum*. The values are given for groups differing by three degrees. The data could, of course, be represented graphically in a curve, but the main point illustrated is brought out quite well from a glance at the figures. They form a series culminating in 120° and ranging somewhat similarly above and below this number. The total number of angles with greater value than

TABLE VII.

FREQUENCIES OF THE ANGLES OF VALUES BETWEEN THE EXTREME 86° AND 176°
IN A SERIES OF THIRTEEN COLONIES OF *P. Boryanum* TAKEN BY CHANCE.
THE VALUES ARE GROUPED BY THREES.

	86° to 88°	89° to 91°	92° to 94°	95° to 97°	98° to 100°	101° to 103°	104° to 106°	107° to 109°	110° to 112°	113° to 115°	116° to 118°	119° to 121°
No. of angles.	1	3	2	2	5	3	14	26	33	42	44	121
	122° to 124°	125° to 127°	128° to 130°	131° to 133°	134° to 136°	137° to 139°	140° to 142°	143° to 145°	146° to 148°	150°	155°	176°
No. of angles.	98	61	39	15	6	4	4	1	2	1	1	1

120° is 252. The total number which have a value less than 120° is 189. The number recorded for 120° is perhaps unduly large. Since, as I have noted, it is quite impossible to judge within one or two degrees with any certainty there is perhaps a tendency to assign too frequently an angle of 120° rather than 119° or 121° . On the other hand, it is not impossible that the angle 120° being the point of equilibrium in the surface tension group, there may be some especial fixity in the configuration when it is once achieved, so that when it arises by chance as a result of the more protracted slow writhing movements of the final stage in forming the colony, it may be maintained in a certain number of points of intersection at the expense even of greater inequalities at other adjacent points. When once attained exactly it may be more persistent than any other angular value. But whether or not the number of angles with a value of 120° is correctly determined there is no question as to the general tendency in these angles to fluctuate about 120° as a center or modal point. On the other hand, it would be quite inadmissible in the light of the results obtained by comparing the corresponding angles of a series of colonies with similar arrangement (Table V.) or a single colony selected for its symmetry ('16, p. 98) to conclude that the type colony of *P. Boryanum* should have all its included angles equal to 120° . With sufficiently large numbers of cases the secondary modal points representing the special values of the included angles of the central cell, etc., should emerge.

The average values for the corresponding sides of the cells in

TABLE VIII.
LENGTH IN MM. OF THE CELL BOUNDARIES IN A SERIES OF SIX
COLONIES OF *P. Boryanum*.
Sides of Central Cells.

Colony.	<i>i.</i>	<i>i</i> ¹ .	<i>i</i> ² .	<i>i</i> ³ .	<i>i</i> ⁴ .	<i>i</i> ⁵ .
87.....	8	7	4.5	5	9	7.5
131.....	9	7	4.5	5.5	7.5	9
1-d.....	11	9	7	6.5	10.5	12
39.....	7.5	9	4.5	4.5	9	7.5
23.....	8.5	9.5	4.5	4.5	8	8.5
55.....	9.5	9.25	6	5	8.75	10.5
Totals.....	53.0	50.7	31.0	31	54.75	55.0
Av.....	8.8	8.4	5.1	5.1	9.1	9.1

Radial Walls of the Cells of Series II.

	<i>k.</i>	<i>k</i> ¹ .	<i>k</i> ² .	<i>k</i> ³ .	<i>k</i> ⁴ .
87.....	7.5	7	7	5	6.5
131.....	7	7	7	6.5	7.5
1-d.....	10	8	9.5	9.5	9.5
39.....	9.5	7.5	8	7	8
23.....	7	6.5	5	5.5	6
55.....	8	9.0	6.5	8.5	8
Totals.....	49.0	45.0	43.0	42.0	45.5
Av.....	8.1+	7.5+	7.1+	7+	7.5

Basal Walls of Cells 7, 9, 11, 13 and 15.

	<i>o.</i>	<i>o</i> ¹ .	<i>o</i> ² .	<i>o</i> ³ .	<i>o</i> ⁴ .	<i>o</i> ⁵ .	<i>o</i> ⁶ .	<i>o</i> ⁷ .	<i>o</i> ⁸ .	<i>o</i> ⁹ .
87.....	7	6.5	6.5	5.5	6.5	6	7	5.5	6.5	4
131.....	4.5	5.5	5	6	5	4.5	5.5	5.5	5	6
1-d.....	7.5	9	7	7	7	6	7	7	7	8
39.....	5.5	6	6	6	5.5	5.5	7	6.5	5.5	6
23.....	5	6.5	6	6.5	5.5	5.5	5	5.5	6	5.5
55.....	7.5	6	6.5	7	7	4	8	7	7	6
Totals....	37.0	39.5	37.0	38.0	36.5	31.5	39.5	37.0	37.0	35.5
Av.....	6.1	6.5	6.1	6.3	6	5.2	6.5	6.1	6.1	5.9

Radial Walls of Series III.

	<i>r.</i>	<i>r</i> ¹ .	<i>r</i> ² .	<i>r</i> ³ .	<i>r</i> ⁴ .	<i>r</i> ⁵ .	<i>r</i> ⁶ .	<i>r</i> ⁷ .	<i>r</i> ⁸ .	<i>r</i> ⁹ .
87.....	6.5	7	7	7	6.5	7	7.5	7	7	7.5
131.....	7.5	7.5	7.5	8	7	8	7	8	7.5	7
1-d.....	8	8	8.5	9	8.5	8		8.5	8.5	7
39.....	7	8	7	8	9	9	8	8.5	8	7.5
23.....	8.5	7	7	7.5	8	8.5	7	7.5	8.5	8
55.....	9.5	10	9.5	8	8.5	9	8.5	8	9	9
Totals....	47.0	47.5	46.5	47.5	47.5	49.5	38.0	47.5	48.5	46.0
Av.....	7.8	7.9	7.7	7.9	7.9	8.2	7.6	7.9	8	7.6

the series of seven colonies of *P. Boryanum* are given in Table VIII., and agree well with those obtained for the selected colony ('16, p. 98). I have not included measurements of the basal walls of cells 8, 10, 12, 14 and 16, as their tendency to equality is sufficiently obvious.

The corresponding radial walls between the cells of series II. tend to be equal as do also the radial walls between the cells of series III. The indication from the measurements of the selected colony that the walls i and i^6 of the central cell should be regarded as typically a little longer than the walls i^1 and i^5 is not confirmed by the measurement of the series, though there is a difference of .2 mm. in the average. Measurements of the dimensions of the central cell in a larger series of colonies would be of interest. Its inherited oblong and two-lobed form, and the pentagonal outline in the colony formed by the bases of the five cells of series II. afford an interesting case of disharmony in morphogenetic factors.

INHERITANCE OF CELL FORM.

I have pointed out that the four-lobed form of the cells appears immediately in the young colonies and have referred to it throughout as inherited rather than as the direct and epigenetic expression of the pressure and other interrelations of the cells in the growing colony. I have pointed out the adaptation of this four-lobed cell form to the exigencies of group formation, when the number of units is strictly limited by the principle of bipartition. The principle of least surfaces here requires that five cells instead of six are to be placed about one in the center and ten cells in the third series. This arrangement involves just such a tangential elongation of the individual cells as we find has actually occurred and favors the maximum of compactness in the arrangement $1 + 5 + 10$. I have suggested the possibility that the environmental complex may have led in successive generations to the development of this four-lobed form and its fixation as an hereditary character of the cells. Evidence that the typical form of the cells can be achieved independently of their being in normal contact and pressure reactions in the colony is rather easy to obtain. Many colonies are found in nature

in which by some accident certain cells are only attached at one point to the remainder of the group.

In Fig. 29 we have an eight-celled colony of *P. asperum* in which one cell, *a*, is attached by only one of its basal lobes and yet is quite symmetrically developed. The second base lobe is rounded rather than sharply wedge-shaped and this difference may be taken as the measure of the influence of the epigenetic pressure and contact relations as compared with heredity in determining the form of the cell. The asymmetric positions of the two central cells in this colony also have produced characteristic effects on their form and it is plain that the relatively free cell has much more nearly achieved its full development than have these

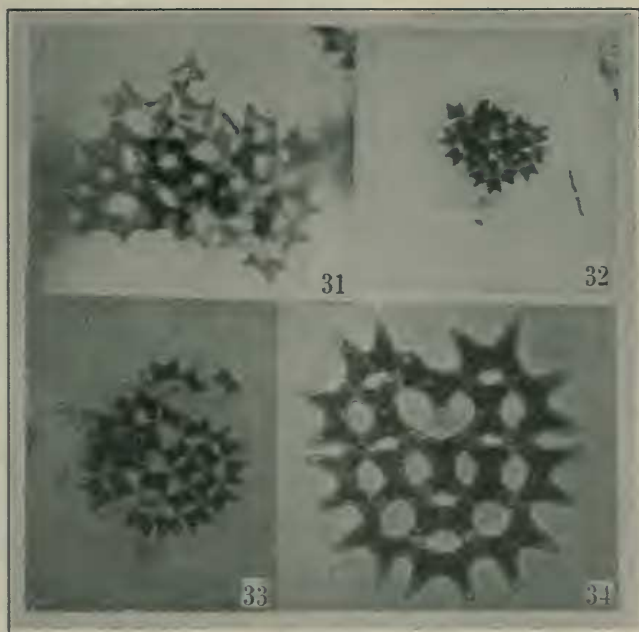


FIGS. 29 AND 30. *P. asperum*. Irregular, eight-celled colonies, one cell in each case attached by one lobe only, but quite typical in outline. $\times$ about 550.

cells with their asymmetric contact relations. In Fig. 30 we have a colony with only seven cells visible, one cell, *a*, attached by only one basal lobe, another by a basal and a peripheral lobe, *b*. It is plain here that in neither case has the free basal lobe tended in any degree to assume the more tapering form of a peripheral lobe, nor have the long and short axes of the cells been reversed. In this colony the eighth cell may have been present in its early life and possibly may have been connected with some of the cells now partly free. The form of cell *b*, however, has certainly been achieved under the same contact conditions in which it appears in the figure. The rounded end of its free basal lobe as well as that of cell *a* shows that the wedge form is an environmental effect.

Figs. 31 and 32 (less highly magnified) also show cells which have attained the normal form while attached by only one basal

lobe. These are probably thirty-two-celled colonies, though they are too irregular to permit accurate counting. The colony shown in Fig. 31 is quite young, while that shown in Fig. 32 is well on toward maturity. The cells attached by only one lobe have gone through their whole development in apparently normal fashion.



FIGS. 31 AND 32. *Pediatrum asperum*. Young colony showing as in Figs. 29 and 30 cells which are without their normal contact and pressure relations in the colony and have still developed the characteristic form for the species. Fig. 31 $\times$ about 500; Fig. 32 $\times$ about 175.

FIG. 33. *P. asperum*. Like the last two figures, but with a cell attached in reversed position by one of its peripheral lobes and still showing the typical form. $\times$ about 225.

FIG. 34. *P. asperum*. Colony with one of its peripheral cells reversed but showing one typical long spine directed toward the center of the colony. The other is blunted by contact with an adjacent cell. $\times$ about 425.

Most interesting is the case shown in Fig. 29, where we have a cell attached only by one of its peripheral lobes and to a peripheral lobe of a peripheral cell of the colony. Here the basal lobes are both peripherally placed and yet have retained their blunter form.

The peripheral lobes are basally placed and one of them functions for attachment and yet they retain their more tapering form.

It is over and over illustrated in abnormal colonies that the polarity shown in the difference between the basal and peripheral lobes is a matter of cell organization and not of colony organization. A very characteristic case is shown in Fig. 34. Here in an otherwise quite regular colony one of the ten peripheral cells has been reversed in position and thrust partly back into the second series. The one free peripheral lobe is quite normally developed though pointing toward the central cell of the colony. The diagonally opposite basal lobe which is free to grow radially outward has shown no tendency to do so. The second true peripheral lobe has had its natural growth tendencies quite inhibited by the limitations of space in which it finds itself. The tendency to functional hypertrophy if operative here is not equal to the production of a normal peripheral lobe under such conditions. The whole grouping in such a case gives a very clear picture of the exact part played by heredity and environment respectively in morphogenetic processes.

Such examples as are shown in Figs. 25-30 can be multiplied almost without limit and it is clear that however much the contact and pressure relations in the group may have influenced the evolutionary processes by which such oval cells as those of *Gonium* become the four-lobed oblong cells of *Pediastrum*, at present these cells are able to attain their characteristic forms, diagnostic for the species, when almost entirely free from their normal environmental relations with the other cells of the colony.

Nitardy figures several marked cases ('14, Taf. VI., p. 2) in which the single spinous outgrowth and general triangular form of the cells of *P. simplex* are shown to be an hereditary growth-habit of the cells rather than a response to their pressure relations of orientation in the colony. In the figure referred to a peripheral cell is shown with its poles reversed and the spine projecting toward the center of the colony and an intercellular space quite as in my Fig. 30 described above.

It would be natural, perhaps, to expect that the four-lobed form should be strictly epigenetic and achieved anew by each generation

under the influence of the stresses and pressures developed in the growing colony. We have noted, however, that the cell form characteristic for the species is visible at once as the swarmspores cease moving and is only sharpened and made more definite with the further growth of the cells. On the other hand, it is equally obvious

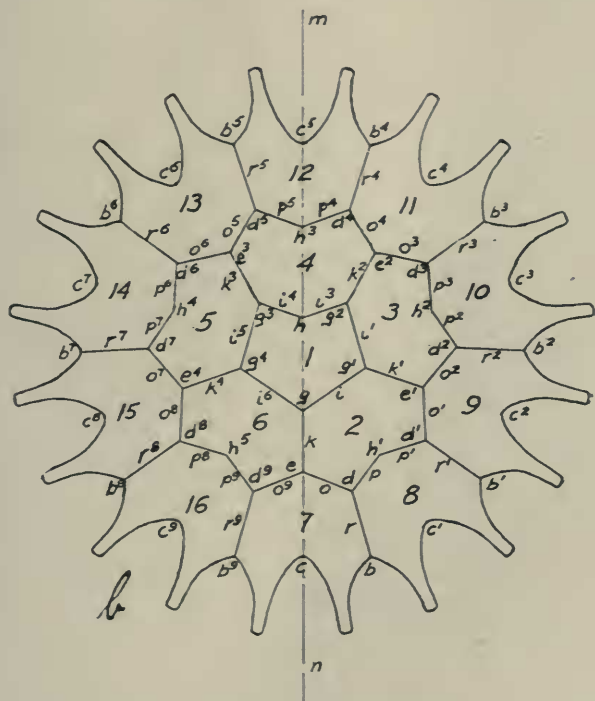


FIG. 35. Type diagram from a selected individual, colony No. 55. Reproduced from '16, Fig. 1b.

that abnormalities of form—shortening, elongation, change of direction of the lobes, etc.—as well as the regular blunting of the peripheral lobes of interiorly placed cells are all direct environmental epigenetic results dependent on the interrelations of contact and pressure between the cells in the forming and growing stages of the colony.

GENERAL DISCUSSION.

Cell Form.—There are plainly two sets of form-determining influences operating on the cells of *Pediastrum*. First, the cell heredity which, given free play, as in the case of cells largely out of contact with other cells of the colony, develops what we may call the typical cell form. And, second, the environmental pressure and contact relations which exist between the cells as ordinarily placed in the colony. This may result in the extreme difference which we find between the internal and peripheral cells of *P. Boryanum* or the very slight differences which we find between these same cells in *P. clathratum* (Fig. 3). The familiar antithesis between heredity and environment in the determination of adult form and structure is fully in evidence in *Pediastrum*, but under such relatively simple conditions as to permit of an attempt at analysis. It is obvious here, as has been held in general by students of heredity, that inheritance through cell division may perpetuate the type form and structure, while many at least of the fluctuating variations in the type are directly traceable to environmental conditions of interaction between the cells and favorable or unfavorable outside conditions at the time the colony is formed and during its growth.

The evidence is clear, as I have shown from the cases of accidentally misplaced cells, that all the various cell forms found in the genus are transmitted from one colony to the next by inheritance in some fashion or other. I shall discuss the method of transmission below. The cell form also obviously determines the character of the colony as a whole. The form and character of the colony as a whole may be said also in turn to influence the form of the cells, but the modifications so produced are of the nature of environmental limitations on the complete development of the cells, as, for example, the shortening of the lobes on the interior cells as compared with the marginal cells of the colony. The position of the cell in the colony influences its form only in minor, though perfectly obvious and definite degrees, but the structural and organic characters of the colonies which are the basis for their classification into subgenera and species are the direct expression of the inherited characters of the cells.

The prevailing oblong, four-lobed form of the *Pediastrum* cell is probably adaptive for the general metabolism of the cells and is also the form which permits the closest possible approximation to a least-surface configuration in a colony composed of units arising by binary fission. It is the form of cell which would be expected to arise under the pressure relations existing in such plates of cells held together by adhesion and yet as noted it can and does arise in cells almost entirely free from such contact and pressure relations. We have here evidence that a cell form which may well have arisen first simply as a response to environmental stimuli has become fixed in heredity until now the series of growth processes by which it develops can go on quite independently of the stimulative conditions which originally called them forth. In *Pediastrum* we do not have the extreme differentiation of germ plasm and soma which under the conditions in the higher metaphytes makes such direct interrelations of environment and heredity so difficult to conceive.

It is sufficiently obvious that the oblong four-lobed form is directly transmitted through vegetative reproduction by cell division and there is no reason to question that the same is true in sexual reproduction by the fusion of gametes. In asexual reproduction the mother cell divides by successive bipartitions to produce a swarm of oval ciliated swarmspores which at first show no trace of their adult form. I have noted, however, how promptly, almost instantaneously, the four-lobed form appears as the swarmers come to rest in the contact and pressure relations of the colony and with the very first growth expansion, so that almost as soon as it is formed the young colony has all the essential structural characteristics of the adult.

We may consider briefly at this point the difficult question as to the method of representation and transmission in heredity of the characters of differentiated tissue cells and the characters of tissues, organs and entire organisms considered as wholes.

Inheritance of Cell Form.—The inheritance of cell form cannot be said to be direct in the sense that the inheritance of green color may be direct. The division of a green cell gives at once two green cells. Greenness is inherited as such by division of the chloroplast

and is a metidentical character in Detto's sense. The color as such may be thought of, at least, as present throughout the whole process of producing the daughter cells from the mother cell. The cylindrical form of a cell of *Spirogyra* may also be inherited directly as such in this same fashion by the division of a cylindrical mother cell into two shorter cylindrical daughter cells.

In the case of the cell of *Pediastrum* the lobed or spinous form disappears in the successive bipartitions of the mother cell and we have an oval ciliated swarmspore essentially similar in form to those of other more or less distantly related green algæ. Reproduction by cell division here has involved the return to what is generally assumed to be a more primitive type of cell both as to its form and its motility. The adult form typical of the species only reappears as a result of ontogenetic development by which the primitive cell form becomes differentiated into the more specialized adult. In all filamentous and cœnobic algæ which reproduce by swarmspores we have this advance beyond the conditions in *Spirogyra*, and related types in which the germ cell differs only in size from the adult. The reproductive cycle in *Pediastrum*, for example, parallels that of the higher plants in its essential stages. A mother cell forms undifferentiated germ cells which become specialized during ontogeny in their form and structure and by their combination produce the many-celled colony which shows also a more or less highly specialized and adaptive organization. In the higher metaphytes it is not so directly obvious as in *Pediastrum* that the form characteristics of the many-celled plant body are the direct expression of the form, polarities, adhesiveness, and other characteristics of the individual cells. The inheritance of cell form and of the form of the colony are indirect as compared with cell color. To be sure, in the latter case swarmspores may be relatively or entirely free from the green color which then reappears in ontogeny but the transfer of the capacity to form green pigment is assumed to involve the division of plastids which thus carry on the pigment-forming bases, chromogens, just as the nucleus, chromosomes, etc., are perpetuated directly by division. In the vegetative reproduction of these simple algæ we do not need to say that the capacity to form chlorophyll is represented by an hereditary factor in the germ plasm,

for we have the visible organ or plastid of the cytoplasm to provide for such transfer. The evidence as to the behavior of the plastids in zygospore formation in *Spirogyra* indicates that the same is true in sexual reproduction by cell fusion. In the case of the lobed cell form, however, every visible trace of the adult character as such seems to be lacking in the germ cell, and it seems natural to postulate a gene or factor which without being the character itself may as a granule or in some other form represent the adult form when it has disappeared. As a matter of fact, however, we have no plastid for form determination and to assume a granule like a plastid in the chromosome which transmits the determiners of the adult cell form meets with obvious difficulties in this case. The sudden appearance of the lobed, spinous cell form in reproduction as I have described it does not suggest the working out of influences emanating from elements in the chromosomal organization of the nucleus, but rather the direct expression of the organization of the cell as a whole when it begins to grow. This organization shows the most direct relations of adaptation to and interdependence with the pressures and contacts established in such a group of cells and may well have been achieved as a response to such environmental surroundings, but it is quite independent of them and comes to full expression, as noted, in cells which are for accidental reasons quite free from the other members of the colony. It seems to me to be most obviously the expression of the anomogenous organization of the protoplasmic mass involving localized growing points on its surface, specific polar differentiations, etc. This general organization of the cell may well be transmitted indirectly through cell division involving as such transmission would only a sort of regeneration by each daughter cell of the general symmetry relations between the parts of the mother cell.

We do not need, then, as it seems to me, to imagine any spatially differentiated organization of a special germ plasma to account for the inheritance of cell form in *Pediastrum*. It would be possible, but probably premature, to attempt to express in diagrammatic form for *Pediastrum* the organization of the cell as a whole which is implied in its behavior in forming the colonies. Much further cytological work such as has been done by Smith on nuclear and cell di-

vision, the centrosome, blepharoplast, plastids, pyrenoids, etc., of *Tetrademus* ('13), *Scenedesmus* ('14), *Characium* ('16), *Pediastrum* ('16), etc., is needed before we shall be able to correlate the evidence for cell polarities, adhesions, growing points, surface tension, etc., with the data from the chemical study of colloids. But it is obvious that it is only on the basis of such studies that we can hope to lay the foundations for a proper theory of the hereditary transmission of characters and the morphogenetic processes by which a mother cell is transformed into a mass of free-swimming swarm-spores and these in turn into the adult colony of *Pediastrum*.

Inheritance of the Characters of the Colony.—The characters of the colony as a whole, however, may seem to involve a much more indirect and hence perhaps properly a factorial representation in the germ cell, but such a conclusion seems to me quite unwarranted. I have elsewhere in a number of cases emphasized the incommensurability of the organization of the cell and that of the many-celled body. Driesch ('05) has shown the impossibility of a preformation which demands even the simplest possible three-dimensional representation of the organization of the adult in the organization of the germ plasm.

The characters of the colony as a whole are dependent, as noted, directly on the form, polarities, adhesiveness, surface tension, etc., of the individual cells. These are characters of the cells as wholes, as protoplasmic aggregates, and there is no reason for conceiving them as especially represented in localized regions of the chromosomes, at least in the case of these simple plants.

We cannot compare cell characters of form with colony characters of form except in the case of some of the simplest surface-tension relations. The colony is an aggregate of cells whose organization is an expression of their cellular interactions in ontogeny. The form characters of the colony may be transmitted down to the details of cell arrangement, shape of intercellular spaces, etc., but these details cannot be conceived as in any way directly represented in a germ plasm.

The complexity of the adult colony is the expression of the differentiation made possible by the interaction of individual cells and their specialization along different lines. An example is found in

the difference between the marginal and interior cells of *P. Boryanum*. The cells all have the same inherited growth tendencies, the differences between them are due to their respective positions in the colony. Such differences are the expression merely of a certain degree of susceptibility to contact and pressure stimuli which, for example, we can think of as greater in *P. Boryanum* than in *P. asperum*. It is certainly more natural to think of such degrees of sensitiveness as characters dependent on the growth reactions of cells as wholes rather than on the presence or absence of a particular chromosomal granule or region.

The organization of the leaf of *Elodea* with its spinous marginal projections affords an interesting parallel in the higher plants. It would be difficult for anyone to conceive that any cell of the leaf might not form a typical spinous projection if it were properly placed on the margin instead of in the interior of the leaf.

We can distinguish then in such cases as those of *Pediastrum* three grades or degrees of directness with which the hereditary transmission of characters is accomplished. First, direct transmission by division, associated with the division of the germ cell, of the particular structural element whose possession constitutes the character, as for example the transmission of green color by chloroplasts. Such a character is metidentical, that is, it is the same thing in the germ cell as in the many-celled organism as a whole. Second, the more indirect transmission of the characters of the differentiated adult cells, which are not visibly present as such in the germ cell. Examples are the lobed form of the adult cells of *Pediastrum*. In this case the conceptions here involved can be easily extended to the higher plants. The stellate pith cells of the bulrush and the elongated thick-walled cells of wood and bast can be thought of as in the same category as to heredity and ontogeny as the lobed cells of *Pediastrum*. Such characters express what we recognize as the organization of the cell as a whole including cytoplasm and nucleus, and are hardly to be conceived as represented in any particular part or organ of the cell. Third, the entirely indirect transmission of the characters of the many-celled organism as a whole, in *Pediastrum*, such characters as the plate-shaped form of the colony, the presence or absence of perforations, the arrangement of the cells,

and the degree of difference between the interior and peripheral cells. It is obvious in *Pediastrum* that such characters are determined directly by the characters of the cells, but it is equally clear that except in their simplest features they are incommensurable with those of the individual cells and cannot be represented directly as such in the germ cell. It is the form, adhesiveness, polarities, etc., of the cells that determine the character of the colony and these characteristics of the cells as noted can best be conceived as characters of the cells as wholes rather than as determined by particular unit parts of cell organs or even by entire cell organs. The general growth tendencies of the cell are characters of the cell not of the colony as a whole and can come to expression regardless of whether the colony is formed or not.

Evidence of Polarity in the Cells.—Furthermore it is obvious as shown above that each cell has a specific orientation in the typical colony, and yet there is no mosaic heredity. Any one cell can replace any other cell in the colony and the shape of the cell is in many species in no notable degree a function of its position or interrelations with the other cells. Each cell in the typical arrangement has its major axis in a definite relation to the major axis of the colony. The long pair of lobes or spines point in general radially outward from the center. This, as I have shown, is not at all because, as one might suspect at first glance, the cell tends to grow its longer spines on whichever side happens to be turned outward. The irregular colonies demonstrate this over and over (Figs 26–28). If in the swarming period the cell does not achieve its normal position with its short axis placed radially and the spine-bearing side outward the maladjustment is never overcome. The long spines push out from the side predestined to produce them and develop as fully as they can under the pressure relations in which they are placed. There is surprisingly little evidence of adaptability in the cells in this regard. Apparently the cell axes are already fixed unalterably in the swarmspore stage and are quite independent of any contact relations then or later established. The presence of a polar differentiation of the axes of the cells in addition to the operation of the principles of binary fission, surface tension, adhesion, functional hypertrophy, etc., is certainly essential in the morphogenetic proc-

cesses by which the plate-shaped colony of one cell layer in thickness and with the longer spines of the cell directed radially outward is formed.

We are confronted here as in practically all higher plant cells with polar differentiation in the cells themselves. The question as to whether this polarity is developed, as is true in so many cases, under the influence of light or gravitation acting during development would seem to be answered in the negative for *Pediastrum*. So far as my observations go, the young colonies are formed in the mother cells at all angles to the direction of the light falling on them and in their orientation to gravitational stimuli. In those cases of rather weak swarming which I have described as forming the oblong instead of circular colonies the shape of the mother cell undoubtedly exerts an influence on the form of the colony. But there is no evidence that the flattened form of the mother cell prevents the swarmspores from making a group of two layers in thickness or an oval mass. The observation of the swarming masses as I have described them suggests most forcibly that, as noted above, the cells actively seek out a position in which they are in equal pressure and contact relations with the adjacent cells right and left of them and with the side which is to develop the large spines or spine radially outward and not vertically upward or downward, involving again in the case of the interior cells contacts with a cell or cells on their basal and peripheral surfaces. No other hypothesis than that of at least a biaxial polarity in the internal organization of the swarmspores seems adequate to account for this definite orientation of the cells in the colony. The existence of the cell axis radial to the colony as a whole is of course most conspicuous owing to the difference in development of the peripheral and basal lobes, but the assumption of a polar differentiation in the tangential axis is equally necessary. The existence of physical polarities or polar differentiations in such complex organisms as the swarmspores with their nuclei, plastids, cilia, etc., is, of course, to be expected. A purely physical factor which may have had phylogenetic significance in the development and fixation of radial polarity is present in the differing lateral pressures to which the basal and peripheral regions of the cells in such a plate-shaped group are subjected. Such differences

in pressure would naturally give a slightly wedge-shaped form to the cells. That this factor is not necessary for the development of the wedge shape of the cell in the formation of the colony now is shown, as I have indicated above, by the fact that the cells will develop their characteristic forms when practically free from contact or pressure relations. The basal and peripheral axial differentiation seems to appear spontaneously now as an expression of the internal organization of the cell whatever may have been its origin, but a slight progressive difference in density from the basal to the peripheral region of the cell may still be one physical condition for such a polarity.

The plate-shaped form of the colony as a whole with one layer of cells plainly depends for its achievement on the transverse or right and left contact relations between of the cells. The cells are so constituted that they achieve contacts with as nearly as possible equal pressures on their right and left sides and, furthermore, so placed that the one or two lobes or spines lie in the plane of these contacts and hence in the plane of the colony as a whole. Such relations can be represented as transverse polarity by the plus and minus or positive and negative analogy, as I have indicated in the colony shown in figure 5. This schematism breaks down in the case of the central cell and for the radial relations of the cells. It is of course only a purely formal representation of the right- and left-sidedness in the cells. There is no visible evidence in the cell itself for the assumption of any structural right and left differentiation. I have found no proof that any particular cell would not find its polarities equally met when rotated through 180° on its radial axis. The only conspicuous evidence of the location of a transverse or tangential axis is in the fact that the spines regularly lie in the plane of the colony.

The assumption of an axis of polarity vertical to the plane of the colony would seem to meet the requirements of the case equally well. The poles would be such in this case as to prevent the cell coming to rest when they were in contact with other cells. However these polar differentiations are conceived it is obvious that some such conditions are necessary for the achievement by free-swimming swarm-spores of a plate-shaped colony of a single layer of cells. That

such morphogenetic factors are organic characteristics of the individual cell rather than the expression of any mysterious form, determining principle residing in the organism as a whole is sufficiently clear, as it seems to me, from the fact that the cell can develop its specific form in entire independence of its interrelations with the other cells of the colony. If, as I think is necessary, one must assume that the cells are characterized by these definite polar differentiations, it becomes still more obvious that the swarming period in its later stages at least when the cells writhe and glide and turn upon each other is not one of aimless movement hither and thither with a final chance distribution of the cells but a definitely directed effort to achieve for each cell a specific relation to its fellows. If the swarmers already had the four-lobed biaxial form we could regard this as a mere matter of physical adjustment analogous to the putting together of the parts of a Chinese puzzle, but as I have pointed out and as earlier students have observed, the cells get their position in the colony as mere oval or egg-shaped jelly droplets. The four-lobed biaxial form appears instantly with the beginning of growth but not before growth begins nor before the cells have their fixed position in the group. It would be highly interesting to know how the cilium-bearing tip, the so-called mouthpiece, of the swarmspore is placed at the moment when the cells come to rest, but I have been unable to determine this point.

It seems to me evident, then, from the shape of the cells and their orientation in the normal colony that they must be regarded as at least biaxial in their relations to each other, the polar differentiations corresponding with their major axes. They are not in equilibrium in their interrelations till the opposite right and left and basal and peripheral poles of their axes are in a general way juxtaposed. In the sixteen-celled colony of *P. asperum* and *P. Boryanum* this would hold for all the cells except the central cell, whose polar relations with the surrounding cells are not easy to analyze. It is perhaps for this reason that the center of the colony is left vacant in the sixteen-celled colonies of *P. clathratum* and the ring-shaped eight-celled colonies of *P. simplex*. The tendency to irregularity in the arrangement of the interior cells of the sixteen-celled colonies of *P. simplex* may also be related to this same difficulty in the polar relations of a central cell in such a group.

Relations of Heredity and Environment to Morphogenesis.—The organization of the colony is determined by the number of the cells resulting always from bipartition, their viscosity, surface tension, mutual attraction and adhesion, their inherited form, whether with one or more spines, and their mutually differentiated polarities by virtue of which they can achieve a definitely oriented relation to each other and thus to the group as a whole. We have here an exhibition of cell qualities influencing morphogenetic processes in such a fashion as to produce a very definite and probably adaptive structural result without any adequate evidence that the characters of the colony as a whole are directly represented in any way in the cells. The form of the colony is achieved in ontogeny as a result of the interactions of the cells.

The characters of the cells are of two classes. First, metidentical as illustrated in the green color, which is transmitted directly by the division of the plastids in which the pigment is borne and, second, characters depending on the organization of the cell as a whole, such as its form, due to its anomogenous consistency from the standpoint of surface tension, its polarities, viscosity, adhesiveness, etc. These cell characters are transmitted in heredity and can be achieved independently of the position of the cell in the colony or any interrelations with other cells. The internal environment of the cell in the colony may modify its form more or less according to the species but is not necessary for its typical development. The close approximation to their normal shape achieved by cells in such irregular colonies as those shown in figures 26, 27, and 28 and in the malformed young colonies (Figs. 21–24) is striking evidence of the fixity of the form characters of the cells as contrasted with the form characters of the colony as a whole. The two types of characters are in different categories.

The influence of the external environment on the form characters of the colony as a whole is most strikingly shown in the differences between colonies in which the swarming has been long continued and vigorous and those in which it has been reduced or has disappeared entirely. There can be no question that in general the colonies of *Pediastrum* are typical in form in direct proportion to the vigor displayed by the swarmspores at the time the colony is or-

ganized. The direct effect of the mother cell vesicle in flattening one or both edges of the plate (Figs. 18-24) in case of slightly weakened colonies is typical of the effect of an unfavorable environment in limiting or inhibiting normal development.

The relative perfection of arrangement of the peripheral and interior cells is also most suggestive of the indirect effects environmental conditions may produce. Statistical studies of the degree of approximation of different colonies to the typical expressed in terms of divergence of cell axes from the axes of the colony as a whole, degrees of distortion of cell form, variation of the angles of intersection of cell walls from 120° , etc., may be expected to afford a reliable index of the effect of environment on the metabolism and growth of the cells.

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DESCRIPTION OF PLATES.

The photomicrographs were made for the most part with the Zeiss apochromatic objective 8 mm. and the compens. oculars, Nos. 8 and 12. They are all of *Pediastrum asperum*.

PLATE V.

Fig. 8. Fairly mature, sixteen-celled colony about ready to form swarm-spores. $\times$ about 225.

Fig. 9. A few cells of a still more mature colony more highly magnified, showing the marked reduction in the size of the intercellular spaces. $\times$ about 1,125.

Fig. 10. Cells *a* and *b*, showing two cell stages, the first cleavage in the short axis of the mother cell. Cells *c*, *d* show at least the beginnings of the four cell stages, the second cleavage plane being in the long axis of the mother cell. In cell *e* the planes are more irregular. $\times$ about 1,125.

Fig. 11. Eight-cell stage, perhaps later, the planes of cleavage still showing a marked tendency to intersect at right angles. $\times$ about 550.

Figs. 12 AND 13. Cleavage complete and the daughter cells more or less rounded up. The whole mass conforms to the outlines of the four-lobed mother cell and there is little evidence of the rectangular intersection of the cleavage planes. Fig. 12 $\times$ about 700; Fig. 13 $\times$ about 1,125.

Fig. 14. Less highly magnified view of a thirty-two-celled colony in all of whose cells cleavage is complete. $\times$ about 500.

Fig. 15. Two young thirty-two-celled daughter colonies and two cells

of the same mother colony just ready to swarm. Empty cells of the same mother colony are also shown. The cells of the two daughter colonies already four-lobed and looking almost as if they were dividing in their short axes. $\times$ about 1,050.

FIG. 16. The same group a few minutes later less highly magnified and showing seven daughter colonies and part of an eighth all more or less out of focus. The swarmspores have just escaped from the right-hand mother cell of the two shown in Fig. 15 and appear as a rounded cloud partly beneath the left-hand mother cell. They are swarming vigorously and are also a little out of focus. $\times$ about 500.

FIG. 17. The same group a few minutes later than the stage shown in Fig. 16. The swarmspores have come to rest in the newly formed colony and the rounded outline of the colony and its concentric series of cells can be made out though it is somewhat out of focus in this figure also. $\times$ about 500.

PLATE VI.

FIGS 18, 19 AND 20. Young daughter colonies still enclosed in the vesicles in which they escape from the mother cell. The vesicles are very transparent and hard to bring out in the photographs. In Figs. 18 and 20 I have traced their outlines over with dilute India ink; in Fig. 19 they are left as they appeared in the print and while they are very faint they can be seen and it is clear that in shape they still maintain the outlines of the mother cell even to the slight projections representing the larger pair of spines. The walls of the mother cells, also shown, enable us to compare the size of mother cell, vesicle and young colony. The tendency of the young colony to conform more or less to the oblong shape of the vesicle is obvious, but the edge view of a colony in Fig. 29 shows that in these cases at least this tendency has not prevented the formation of the plate of a single layer of cells though the space relations in the vesicle would favor the formation of two or three layered groups such as are shown in Fig. 23. $\times$ about 1,050.

FIG. 21. Two young daughter colonies, one with sixteen, the other with thirty-two cells, both being the offspring of a thirty-two-celled mother colony. The young colonies are of the same age, but the cells of the sixteen-celled colony are proportionally larger. $\times$ about 500.

FIG. 22. A mother colony and six young daughter colonies, all of which show more or less the influence of the oblong mother cell vesicle on their shape. $\times$ about 150.

FIG. 23. Ten extremely young daughter colonies, all from the same mother colony. Reproduction occurred in this case after the mother colony had been sealed up as described for about eighteen hours and the free swimming movements of the swarmspores were almost entirely suppressed. The colonies are two or more layers thick and the interrelations of the cells are entirely abnormal and yet the cells themselves have taken on the four-lobed form typical of the species. $\times$ about 150.

FIG. 24. Four young colonies from the same mother colony, illustrating still further, as do the preceding figures 15 to 23, the extreme range of fluctuating variation in cell arrangement which can be found in the offspring of a single mother colony, while in all the type of cell form remains quite constant. $\times$ about 400.